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PLoS GENETICS

Complex Evolutionary Events at a Tandem Cluster of *Arabidopsis thaliana* Genes Resulting in a Single-Locus Genetic Incompatibility

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Abstract

Non-additive interactions between genomes have important implications, not only for practical applications such as breeding, but also for understanding evolution. In extreme cases, genes from different genomic backgrounds may be incompatible and compromise normal development or physiology. Of particular interest are non-additive interactions of alleles at the same locus. For example, overdominant behavior of alleles, with respect to plant fitness, has been proposed as an important component of hybrid vigor, while underdominance may lead to reproductive isolation. Despite their importance, only a few cases of genetic over- or underdominance affecting plant growth or fitness are understood at the level of individual genes. Moreover, the relationship between biochemical and fitness effects may be complex: genetic overdominance, that is, increased or novel activity of a gene may lead to evolutionary underdominance expressed as hybrid weakness. Here, we describe a non-additive interaction between alleles at the *Arabidopsis thaliana OAK* (*OUTGROWTH-ASSOCIATED PROTEIN KINASE*) gene. *OAK* alleles from two different accessions interact in F_1 hybrids to cause a variety of aberrant growth phenotypes that depend on a recently acquired promoter with a novel expression pattern. The *OAK* gene, which is located in a highly variable tandem array encoding closely related receptor-like kinases, is found in one third of *A. thaliana* accessions, but not in the reference accession Col-0. Besides recruitment of exons from nearby genes as promoter sequences, key events in *OAK* evolution include gene duplication and divergence of a potential ligand-binding domain. *OAK* kinase activity is required for the aberrant phenotypes, indicating it is not recognition of an aberrant protein, but rather a true gain of function, or overdominance for gene activity, that leads to this underdominance for fitness. Our work provides insights into how tandem arrays, which are particularly prone to frequent, complex rearrangements, can produce genetic novelty.

Citation: Smith LM, Bomblies K, Weigel D (2011) Complex Evolutionary Events at a Tandem Cluster of *Arabidopsis thaliana* Genes Resulting in a Single-Locus Genetic Incompatibility. PLoS Genet 7(7): e1002164. doi:10.1371/journal.pgen.1002164

Editor: Rodney Mauricio, University of Georgia, United States of America

Received October 20, 2010; **Accepted** May 17, 2011; **Published** July 14, 2011

Funding: This work was supported by European Community FP7 Marie Curie Fellowship PIEF-GA-2008-221553 and an EMBO Long-Term fellowship (LMS), European Community FP6 IP AGRON-OMICS (contract LSHG-CT-2006-037704), a Gottfried Wilhelm Leibniz Award of the DFG), and the Max Planck Society (DW). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Both evolutionary biologists and breeders have long been interested in non-additive interactions among alleles at the same locus. For example, explanations for heterosis or hybrid vigor, a staple of modern agriculture, share many conceptual formalities with models proposed by Bateson, Dobzhansky and Muller to explain how negative heterosis could result from two or more genes that accumulate different changes in separate lineages. The associated phenotypes of hybrid weakness, sterility or lethality in turn may ultimately lead to reproductive isolation and hence speciation ([1–3], reviewed in [4,5]). Hybrid incompatibilities form a continuum from the grey zone of developmental abnormalities through the clearer phenotype of F_1 sterility to the severest form, lethality, and it is important to understand the genetic and molecular causes for the entire spectrum of incompatibilities.

F_1 incompatibilities have been found in as many as 2% of *Arabidopsis thaliana* intra-specific hybrids [6]. Several similar cases in *A. thaliana* and other species involve interactions between alleles of disease resistance genes with other loci in the genome, which cause an autoimmune syndrome known as hybrid necrosis [6–8]. That hybrid necrosis is such a relatively common phenomenon is easily explained, since genes involved in plant defense are highly variable between different individuals of the same species [9,10], and thus make a perfect substrate for causing problems when different genomes are combined. Moreover, several important classes of defense genes, including those encoding nucleotide binding-leucine rich repeat (NB-LRR) proteins and receptor-like kinases (RLKs), commonly occur in tandem arrays, and new alleles are easily created through gene duplication, illegitimate recombination and gene conversion [11–19].

In addition to inappropriate activation of the immune system or sterility, aberrant development is often observed in incompatible plant hybrids [20,21]. Both *Triticum* and *Nicotiana* interspecific hybrids frequently suffer from tumor-like tissue proliferation [22,23]. In *Nicotiana* hybrids, wounding and physiological stresses enhance tumor formation, and tumors may differentiate into recognizable tissues [24]. Genetically-induced tumors have also

Author Summary

While intraspecific hybrids are vitally important in modern agriculture because they often perform better than their inbred parents, certain hybrid combinations fail to develop normally and are inferior to their parents. We have identified an *Arabidopsis thaliana* hybrid with several aberrant growth phenotypes that are caused by divergence at a single locus encoding the receptor-like kinase *OUTGROWTH-ASSOCIATED PROTEIN KINASE* (*OAK*). *OAK* belongs to a group of similar genes arranged in a tandem cluster that varies substantially between *A. thaliana* strains. *OAK* originated through duplication within the cluster with concurrent recruitment of coding sequences from nearby genes to form a new promoter with a novel expression pattern. Kinase activity of OAK is required for its effects, indicating that it is not recognition of an aberrant protein but rather a true gain of function that leads to the incompatibility. Most of the incompatibility seems to come from divergence within the extracellular ligand-binding domain of the OAK protein, indicating that heterodimers of OAK may have higher affinity for a natural substrate compared to either homodimer. Finally, mis-expression of the incompatible *OAK* alleles from the promoter present in the reference strain of *A. thaliana* also leads to genetic incompatibility, but with different phenotypic outcomes.

been described in hybrids of *Brassica*, *Datura*, *Solanum* and *Lilium* [24]. Developmental abnormalities in intra-specific moss hybrids have recently been linked to putatively structurally divergent regions [25], similar to the association of hybrid necrosis with structurally diverse disease resistance loci.

While the known cases of F_1 hybrid incompatibility are mostly caused by interaction between alleles at unlinked loci, of particular interest are situations of heterozygous advantage (overdominance) or disadvantage (underdominance) due to interaction of divergent alleles at the same locus. Overdominance has been advanced as an important contributor to hybrid vigor, or heterosis [26–28]. Conversely, underdominance may underlie hybrid weakness, sterility or lethality, and thus contribute to speciation [20,21,29]. It should be noted that cases of heterozygous disadvantage are underdominant with respect to fitness but can be overdominant in the genetic sense: a plant may become less fit due to increased activity of the gene(s) involved.

Although evidence for both single-gene over- and underdominance is easily found in whole-genome expression studies (e.g. [28]), few cases with phenotypic consequences are understood at the molecular level. Schwartz and Laughner [30] reported four decades ago an example in maize, where two partially compromised forms of alcohol dehydrogenase can form a fully active homodimer; a similar case has been described for complementing alleles at the ARF GTPase-encoding *GNOM* locus of *Arabidopsis thaliana* [31]. In tomato, a heterozygote for a loss-of-function allele of the *SFT* gene has increased yield [32]. Finally, a particularly revealing study comes from rice, where sterility ensues when two divergent alleles at the *S5* locus are combined [33]. Since this is not observed when either allele is heterozygous with a third, presumably non-functional allele, one can infer that the combination of the two *S5* alleles results in gain-of-function activity of the encoded aspartate protease. The *S5* interactions thus provide an example of the complex relationship between biochemical and fitness effects, as the underdominant fitness effects are not simply a consequence of reduced gene activity. It also provides a counterpoint to the *SFT* case, where reduced gene activity has overdominant fitness effects [32].

Here, we report on an intraspecific *A. thaliana* F_1 hybrid, where heterozygosity at a single locus causes a pleiotropic syndrome that includes smaller stature and reduced seed set as well as ectopic outgrowths on leaf petioles. The causal receptor-like kinase (*RLK*) gene, *OUTGROWTH-ASSOCIATED PROTEIN KINASE* (*OAK*), is found in a structurally hypervariable tandem cluster of related *RLK* genes. During duplication of the ancestral *RLK* gene, coding sequences were recruited to form a promoter with a new expression domain. Divergence in the extracellular domain of the protein led to evolution of alleles that now interact in the Bla-1/Sha hybrid to produce phenotypes not seen in the parents, making this a case of underdominance for fitness caused by overdominance for gene expression.

Results

Ectopic petiole outgrowths and reduced biomass of Bla-1/Sha hybrids

The aberrant phenotype of Blanes-1 (Bla-1)/Shahdara (Sha) F_1 hybrids was identified in a survey of more than 1,300 crosses among over 300 *A. thaliana* accessions from the world-wide range of the species [6]. Bla-1/Sha F_1 plants had a range of phenotypes that were not normally seen in inbred accessions, including the Bla-1 and Sha parents, or in other F_1 hybrids: outgrowths on the adaxial surface of the petioles, leaf twisting, leaf lesions, and loss of apical dominance reflected by precocious and increased release of side shoots (Figure 1a–1c). These phenotypes were observed regardless of the direction of the cross. Raising plants in long days at 23°C instead of 16°C restored apical dominance and largely suppressed leaf twisting and lesioning. This partial suppression of the hybrid phenotype at higher temperatures is similar to the suppression of necrosis seen in the Uk-1/Uk-3 and other hybrids with autoimmune defects [6].

Because the ectopic outgrowth phenotype was particularly striking and reliably observed in all F_1 plants, we decided to investigate it in detail. The same phenotype with little variation was seen in approximately 50% of all F_2 progeny, compatible with a single-gene, heterozygous genetic basis. The outgrowth phenotype segregated independently of the lesioning in the F_2 and subsequent generations.

Outgrowths were occasionally noted in the Bla-1 parent, but with incomplete penetrance that varied greatly between experiments (Table S1). Onset of outgrowth formation in Bla-1, when it occurred, was much later than in the F_1 hybrids. Crosses of each parental line to the reference accession Col-0 did not produce any progeny with outgrowths, but they were, as expected, seen in about one quarter of progeny after Col-0/Bla-1 and Sha/Col-0 F_1 hybrids were crossed to each other.

Analysis of transverse sections revealed that outgrowths originated from proliferating parenchyma and/or epidermal cells on the adaxial surface of the petiole (Figure 1d–1f). The vascular system of the petioles appeared normal. Because of their determinate nature, we concluded that the outgrowths did not constitute undifferentiated callus.

We also asked whether the gene(s) causing the hybrid phenotypes of outgrowth and lesioning might affect overall plant performance. In a segregating F_2 population of five-week old plants, we found that outgrowths alone were correlated with a 29% reduction in rosette weight, while lesioning or lesioning plus outgrowths reduced growth by over 50% (Table S2; 2-way ANOVA outgrowths $p = 0.0003$, lesioning $p<0.0001$). In addition, we assessed seed set as a proxy for lifetime fitness. Due to confounding factors such as differential flowering times in Sha and Bla-1, we measured seed set after the incompatibility was

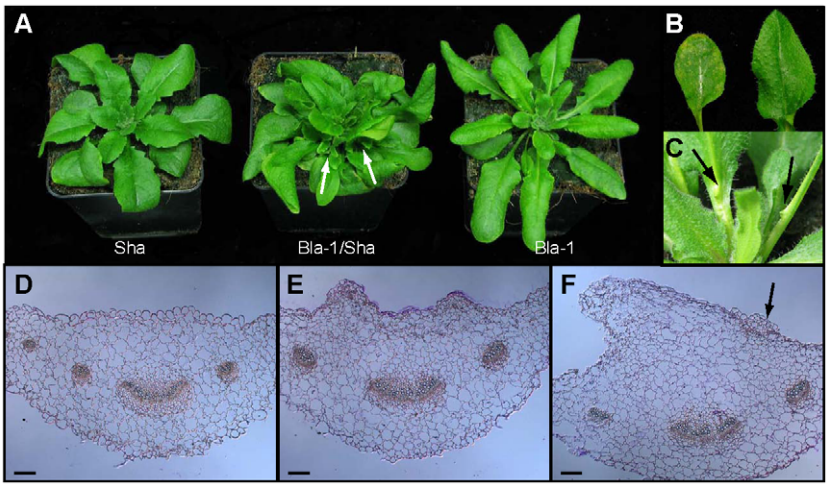


Figure 1. Adaxial outgrowths in Bla-1/Sha hybrids. (a) Six-week old plants grown at 16°C, long days, of Sha (left), Bla-1/Sha F_1 hybrid (centre) and Bla-1 (right). Arrows indicate de-repressed side shoots in the hybrid. (b) Lesioning is seen in leaves of six-week old F_2 hybrid plants grown at 16°C, long days, where the phenotype segregates (present on left leaf, absent on right leaf). (c) Outgrowths on the petioles of Bla-1/Sha F_2 plants grown at 23°C, long days. (d–f) Transverse sections of Bla-1 (d) and Bla-1/Sha hybrid (e, f). Outgrowths that are caused by proliferation of parenchyma and/or epidermal cells are visible on the adaxial surface of the petiole. Scale bar = 100 μm.
doi:10.1371/journal.pgen.1002164.g001

reconstituted in the Col-0 reference background (see below for further details). Seed set was reduced by 90% in F_1 hybrids that were phenotypically comparable to the natural hybrids (two-tailed, unequal variance t-test: $p<<0.001$; Figure S1). In two other independent crosses that resulted in a more severe incompatibility phenotype, all the hybrids died within two months, and thus did not produce any seeds at all. This indicates that the Bla-1/Sha OAK incompatibility greatly reduces lifetime fitness.

Because wounding and physiological stresses enhance the formation of tumors in *Nicotiana*, where these may differentiate into recognizable tissues [24], we examined the effects of wounding, by pricking the petioles of Bla-1/Sha F_1 plants with a fine needle. Outgrowth formation was not enhanced, but we found that increased humidity suppressed outgrowth formation (Figure S2). This is reminiscent of the suppression of constitutive activation of disease resistance in the *ssi4* mutant by high humidity [34].

Compared to normal tissue, induction of callus from *Nicotiana* hybrid tumors requires less auxin [35]. Some *A. thaliana* tumor forming lines also produce callus tissue that can continue to proliferate on hormone-free media [36]. To test auxin response in our system, transverse sections of leaf and petiole tissue were induced to form callus. Although the Bla-1 parent had a relatively higher auxin requirement for callus formation, there was no difference between the Sha parent and the Bla-1/Sha hybrids (Figure S3). Thus, the outgrowths are probably genetically distinct from the *Nicotiana* tumors.

Genome-wide expression studies

Microarray analysis with triplicate Affymetrix ATH1 arrays using RNA extracted from three-week-old aerial tissue identified 356 genes differentially expressed in the hybrids compared to the parents. There was no significant up- or down-regulation of any particular known pathways or reactions based on the SkyPainter tool [37], but several, often overlapping, Gene Ontology (GO) categories were enriched among the differentially expressed genes, most notably several related to pathogen response (Table S3; [38]). Whether this reflects a link to disease resistance remains unclear, since some well-known markers for pathogen response, such as *PR1* or the defensin gene *PDF1.2(b)*, were down-regulated in the hybrids (Tables S4 and S5). In any case, as with the morphological phenotype, there was no overwhelming connection to the hybrid necrosis syndrome as seen in many other incompatible *A. thaliana* F_1 hybrids [21].

Ectopic outgrowths caused by a hypervariable protein kinase gene cluster

Using F_2 and F_3 progeny, we mapped the outgrowth phenotype to a single genomic region on chromosome 5 containing 17 genes in the reference accession Col-0 (At5g59560 to At5g59700; Figure S4). A tandem array of four genes that encode a distinct clade of closely related receptor-like kinases (RLKs; At5g59650 to At5g59680) [17] were of particular interest, because RLKs are one of the most variable gene families in the *A. thaliana* genome [9].

We recovered the genomic regions from At5g59616 (encoding a protein kinase-related protein) to At5g59690 (histone H4) by long-range PCR from Bla-1 and Sha, and found the *RLK* cluster to be highly variable (Figure 2a). In Col-0 only, there are two transposons and a pseudogene upstream of the *RLK* genes. In Sha, the first *RLK* gene in the cluster, At5g59650, is missing and the upstream gene At5g59616 is only partially present. In both Bla-1 and Sha, a 150 bp remnant of the second *RLK* gene, At5g59660, indicates that a deletion likely occurred in the Bla-1/Sha lineage. Also in both Bla-1 and Sha, the third *RLK* gene of the cluster, At5g59670, has been duplicated to give rise to At5g59670a and At5g59670b (Table S6). In addition to Bla-1 and Sha, the At5g59670 duplication was detected by PCR analysis of the *OAK* promoter in 36 of 87 diverse *A. thaliana* accessions (Table S7), while a Col-0 like promoter was found in 45 accessions. Assays for both promoter types were positive in two accessions, indicating either illegitimate recombination or a different duplication event. The PCR assays failed in the remaining four accessions.

Reconstruction of the ancestral state of the tandem array, by comparison with the close relative *A. lyrata* [39], suggested the presence of three tandem *RLK* genes in the last common ancestor of *A. thaliana* and *A. lyrata*. The central gene was duplicated in the *A. thaliana* lineage to produce At5g59660 and At5g59670, whereas in *A. lyrata*, there have been subsequent duplications of the two flanking *RLK* genes, resulting in a cluster with six genes. Given the

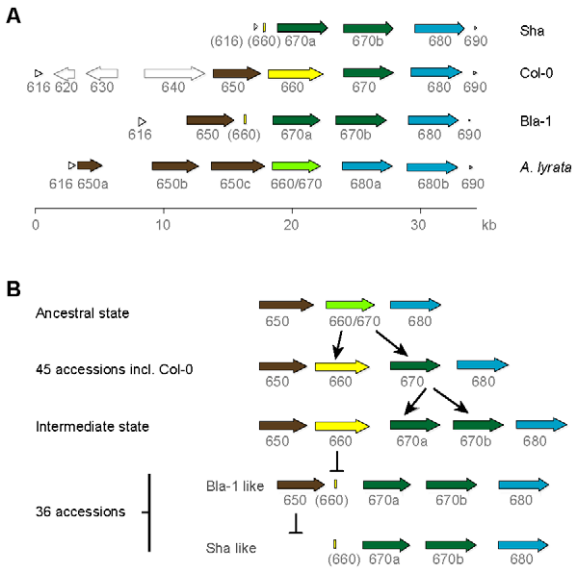


Figure 2. OAK kinase cluster architecture. (a) Last three digits of At5g59XXX gene identifier given. Truncated genes are indicated by brackets around the gene identifier. CACTA transposons and the pseudogene in Col-0 are indicated by light grey, unfilled arrows. (b) Hypothesized events in the evolution of the OAK kinase cluster in Bla-1 and Sha.
doi:10.1371/journal.pgen.1002164.g002

presence of a remnant of At5g59660 in Bla-1 and Sha and that the Col-0-like At5g59670 is found in over half the accessions tested, the ancestral state of this cluster in *A. thaliana* is likely to have been a cluster of four *RLK* genes as found in Col-0 (Figure 2b).

Two alleles of a single *RLK* cause novel growth phenotypes

To determine whether any of the *RLK* genes contribute to the outgrowth phenotype, a genomic copy of each gene from Bla-1 and Sha was individually introduced into the Bla-1, Sha and Col-0 backgrounds. Only plants transformed with At5g59670b from Bla-1 or Sha developed outgrowths (Figure 3a). Unexpectedly, while At5g59670b from Bla-1 induced outgrowths most effectively in Sha, and At5g59670b from Sha in Bla-1, outgrowths were also seen, albeit at lower frequency, upon transformation of either gene into the recurrent parent or into Col-0. This suggests a dosage effect, perhaps due to elimination of negative regulatory elements or epigenetic marks in the transgene that normally suppress expression of the endogenous locus, such that the transgenic proteins are present at an elevated level compared to native OAK_{Sha} or OAK_{Bla-1}. This is supported by some transgenic lines in which we saw a 3:1 ratio of normal to affected plants in the T_2 generation, such that a hemizygous state gives a wild-type phenotype while homozygosity for the transgene leads to a Bla-1/Sha-like phenotype. A similar increase in incompatibility severity after transgenic reconstitution was also observed for *DM1* in the case of Uk-1/Uk-3 [6].

To determine whether the *RLKs* were not only sufficient, but also necessary for the outgrowths, artificial miRNAs (amiRNAs) were designed against individual *RLKs* [40]. Only Bla-1/Sha plants with an amiRNA directed against At5g59670b showed a suppression of the hybrid phenotype (outgrowths, leaf twisting and apical dominance; Figure 2b and Figure S5). We therefore refer to At5g59670b as *OUTGROWTH-ASSOCIATED PROTEIN KINASE* (*OAK*).

Comparison of Bla-1 and Sha *OAK* alleles

The Bla-1 and Sha *OAK* primary transcripts are each 3.9 kb long, with 13 exons, and a 5′ untranslated region of 92 nt (expressed in Bla-1 and Sha petioles) or up to 123 nt (expressed in Sha pedicels and peduncles), as determined by 5′ RACE-PCR. Both *OAK* alleles encode proteins of 873 amino acids, with 9% of residues being different. The majority of polymorphisms are located in a 152 amino acid region, between positions 180 and 331, where 55 residues differ (Figure 3c). Among the remaining 721 residues, there are only 19 replacements.

Like many other plant RLKs, the OAK proteins include a signal peptide, potential leucine-rich repeats (LRRs; in OAK, four to five), a transmembrane domain, and a cytoplasmic kinase domain (Michael Hothorn, personal communication; Figure 3d and Figure S6). In addition, two related regions with similarity to a carbohydrate-binding domain in ER-localized malectin proteins from animals [41] are found between the signal peptide and the LRRs (http://toolkit.tuebingen.mpg.de/hhpred/; Michael Hothorn, personal communication). Interestingly, the region that is very different between the Bla-1 and Sha proteins, from residue 180 to 331, coincides almost perfectly with the second predicted malectin-like domain, from residue 169 to 331. An analysis of OAK and its homologs (OAK_{Sha}, OAK_{Bla-1}, $At5g59670a_{Sha}$, $At5g59670a_{Bla-1}$ and $At5g59670_{Col-0}$), using the *Codeml* program of PAML to assess dN/dS ratios, did not provide evidence for directional or diversifying selection across the entire protein [42,43]. However, an Bayesian Posterior Probability analysis of positive selection at individual residues, using $At5g59670_{Col-0}$ as a reference, suggested that several codons in the second malectin-like domain are under positive selection [44]. A broader analysis of 34 accessions from which *OAK* sequences could be recovered supported these conclusions (Figure 3d).

To determine if the second malectin-like region in OAK homologs is generally hypervariable, we performed a sliding window analysis of all eleven RLKs in the Col-0, Bla-1 and Sha clusters (Figure S7). Most highly conserved are the LRR and kinase domains. We also examined in detail the duplicated genes encoding the At5g59670 proteins. $At5g59670a_{Sha}$ and OAK_{Sha} stood out, because they are identical across the first 598 amino acids of the protein. At the nucleotide level, the two genes include an identical 2.7 kb fragment, which most likely reflects a recent gene conversion event that extends from 13 bp upstream of the translational start site to the first 60 bp of the kinase encoding sequences. In conclusion, the divergence between the second malectin-like domain of OAK_{Bla-1} and OAK_{Sha} is not representative of the variation between RLKs encoded by orthologs and paralogs in this cluster.

Role of divergent promoter sequences in causing the *OAK* hybrid phenotype

To determine the contribution of non-coding and coding sequences of *OAK* to the outgrowth phenotype, we performed a series of domain swaps between OAK_{Bla-1}, OAK_{Sha}, and $At5g59670_{Col-0}$ (Figure 4a). Similar to plants transformed with the non-chimeric fragments, T_1 transformants frequently showed more severe phenotypes than were observed in the F_1 hybrids. This indicated that divergent *OAK* alleles have the potential to cause even stronger incompatibilities than seen between the accessions Bla-1 and Sha.

The first major conclusion from the experiments with the chimeric transgenes was that the promoter region contributed to the outgrowth phenotype, because outgrowths were only observed when a particular recombinant protein was expressed from either the OAK_{Bla-1} or OAK_{Sha} promoter, but never with the

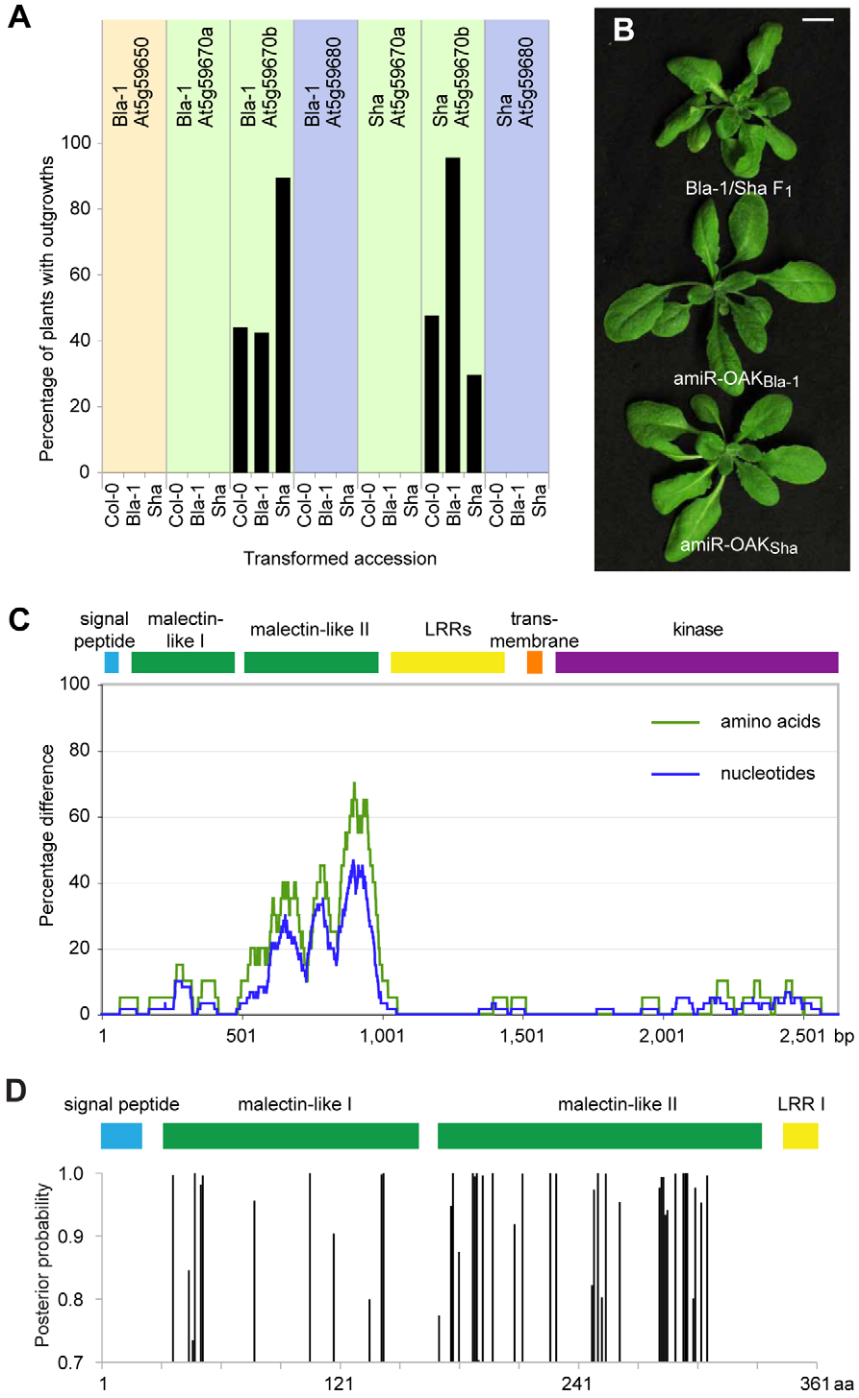


Figure 3. Identification of At5g59670b homologs as sufficient and necessary for outgrowths. (a) Fraction of T_1 plants (n≥90, except for Bla-1 transformed with Sha At5g59680 where n = 56) with outgrowths. (b) Suppression of outgrowths with amiRNAs against *OAK* (At5g59670b) from Bla-1 or Sha. (c) Divergence between *OAK* (At5g59670b) alleles from Bla-1 and Sha (sliding windows of 60 bp and 20 amino acids, respectively). (d) Identification of individual sites in the N-terminal part of OAK protein under positive selection (as determined by Bayesian Posterior Probability) across 34 accessions using PAML [43]. The second malectin-like domain is enriched for such sites.
doi:10.1371/journal.pgen.1002164.g003

$At5g59670_{Col\text{-}0}$ promoter (Figure 4b). GUS reporter experiments demonstrated that the *OAK* promoters from Bla-1 and Sha were active in the vascular system of the petioles, in a pattern consistent with the location of the outgrowths (Figure 5). In contrast, the $At5g59670_{Col\text{-}0}$ promoter drove expression in the leaf lamina, explaining why it could not cause petiole outgrowths. The activity domain of the $At5g59670a_{Bla\text{-}1}$ promoter was similar to that of the $At5g59670_{Col\text{-}0}$ promoter, but with additional expression in the lamina of the cotyledons. Finally, the $At5g59670a_{Sha}$ promoter was active in all seedling tissues, but in isolated patches that differed from plant to plant. Thus, despite the encoded proteins being closely related, the promoters

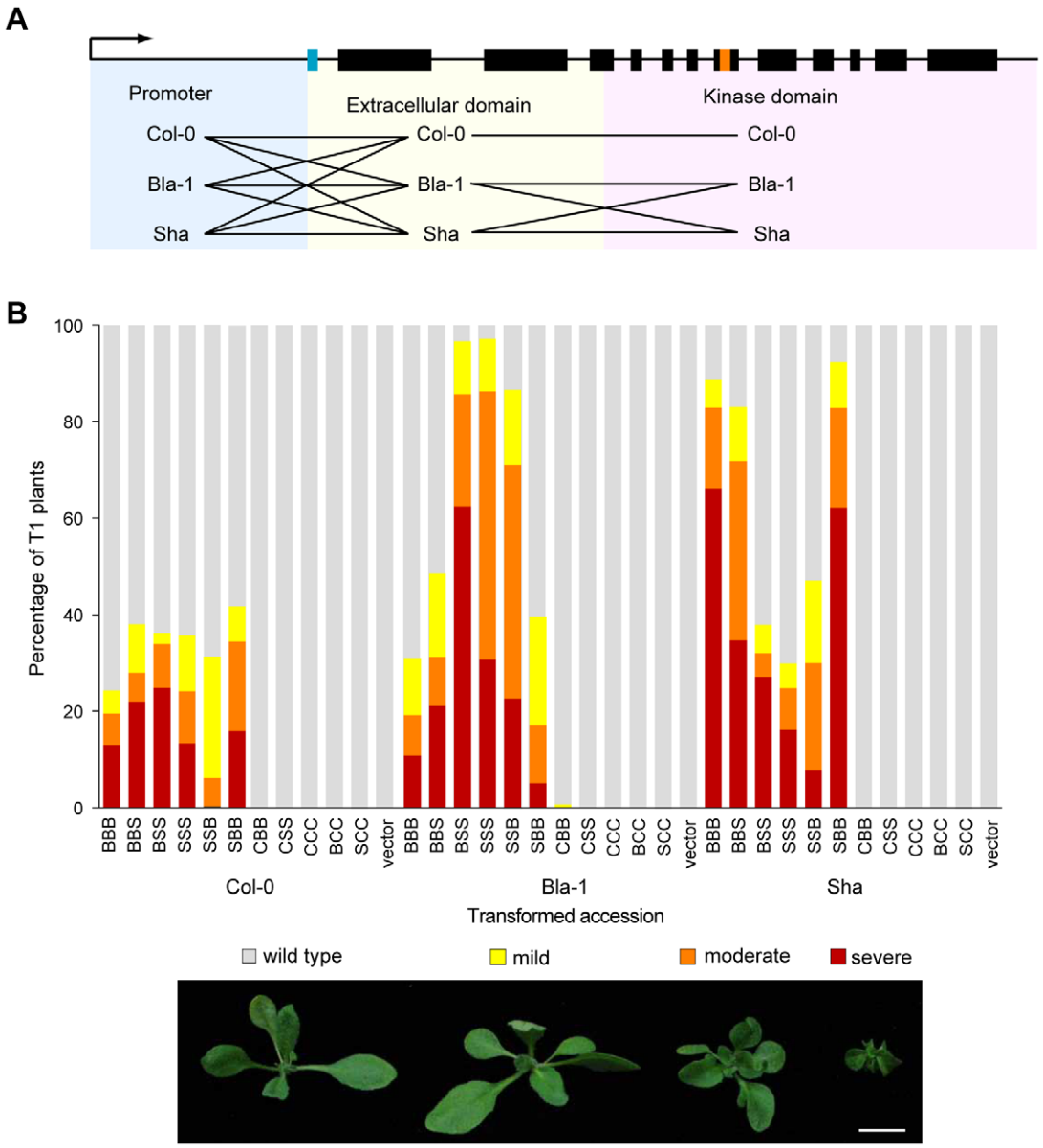


Figure 4. Contribution of both the *OAK* promoter and extracellular domain to outgrowths. (a) Overview of domain swaps. (b) Phenotypic distribution of T_1 plants (n≥90). Three-letter code indicates composition of chimeras. E.g., BBS, promoter and extracellular domain from Bla-1, kinase domain from Sha. Examples of phenotypic classes are shown at the bottom: mild (outgrowths, but otherwise normal leaves), moderate (outgrowths, shortened petioles, mild leaf twisting, normal lamina size) or severe (stunted plants, petioles almost absent, reduced lamina surface, seed rarely obtained). Scale bar = 1 cm.
doi:10.1371/journal.pgen.1002164.g004

conditioned a surprisingly wide spectrum of expression patterns, with differences both between duplicates within an accession and among orthologs from different accessions.

Diversity and origin of promoters in the *OAK* cluster

The $OAK_{Bla\text{-}1}$ and OAK_{Sha} promoters are more similar to each other than are the coding regions, being 97% identical in the 1,238 bp upstream of the start codon. *OAK* promoter sequences could be recovered from a further 32 accessions. Pairwise identity for all 34 accessions including Bla-1 and Sha was between 97 and 100%. Given the high similarity of the promoter region, the duplication of At5g59670 to form *OAK* is unlikely to have occurred more than once. Therefore while the change in expression domain has determined how the incompatibility is expressed, the causative changes for the incompatibility are not within the promoter region. In comparison, over the first 1,077 bp of the coding region, the pairwise identity for the 34 accessions ranged from 87 to 100%, with a mean of 94%. One accession that was identical to Sha throughout both the promoter and coding region was Kondara, which we found to be incompatible with Bla-1 as well. Across the entire *RLK* cluster, there were only two nucleotide differences in 17.5 kb, and both were in non-coding sequences. Kondara was therefore not considered separately in any of the sequence analyses. Further crosses of Bla-1 and Sha to other accessions with the *OAK* gene revealed that while most accessions are compatible, a similar

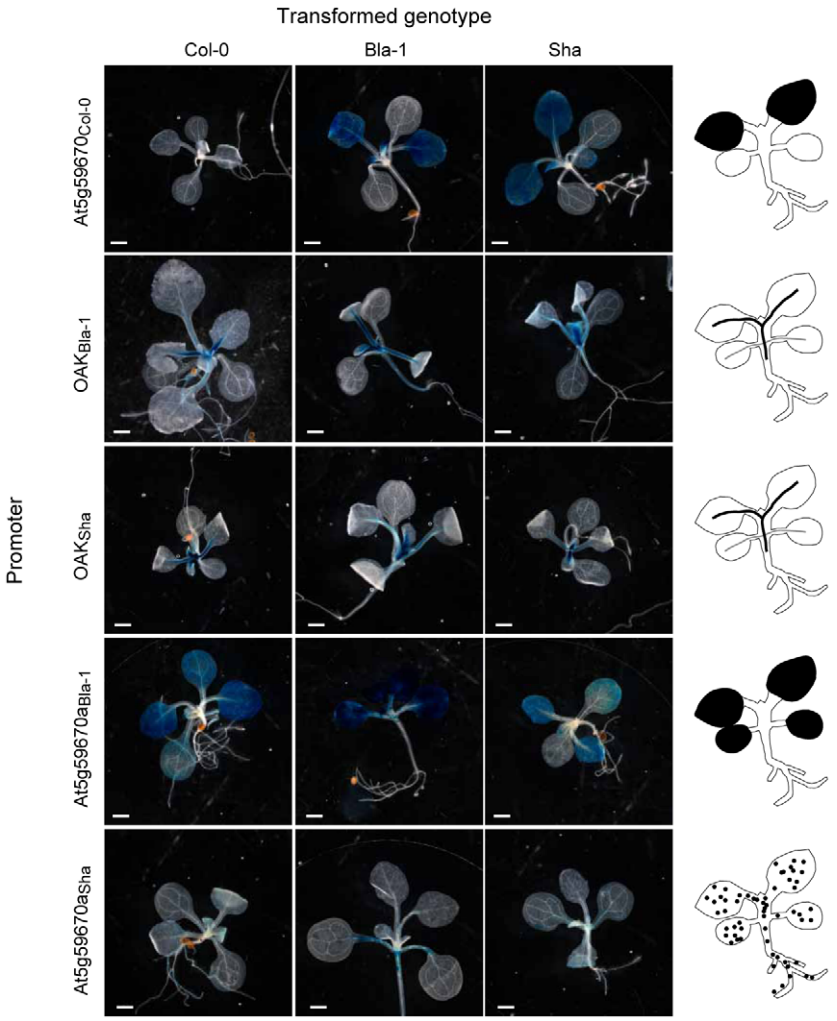


Figure 5. Activity domains of *OAK* homolog promoters. A representative T_1 plant for each promoter:GUS construct transformed into Col-0, Bla-1 and Sha is shown, with diagrams of the expression domain on the far right. Scale bar = 100 μm.
doi:10.1371/journal.pgen.1002164.g005

incompatibility phenotype is seen in Sha x Bak-2, Sha x Leo-1, Mer-6 x Bla-1 and Leb-3 x Bla-1 hybrids (all incompatibilities between Bla-1-like and Sha-like haplotype groups based on the second malectin domain; Figure S8). Less severe incompatibilities with a late onset of outgrowth formation were found in crosses of Bla-1 to a number of accessions with a second malectin domain that fell into a different haplotype group (ICE91, ICE92, ICE152, ICE153, Vash-1 and Valsi-1).

Using NeighborNet implemented in SplitsTree [45], we examined the relationship between the *RLKs* from the 34 accessions based on the promoter sequences and the extracellular domains (amino acids 1 to 360; Figure 6a, 6b). Similarity in the coding region was not always reflected in promoter similarity, and vice versa, suggesting a history of recombination or gene conversion events. The SplitsTree analysis suggested four major haplotypes at the *OAK* locus. Analysis with STRUCTURE [46], where we treated polymorphisms in the *OAK* locus as linked markers on a chromosome, confirmed that there are four major haplotype groups, with half of the accessions studied showing contributions from more than one haplotype (Figure 6c). Within-locus switching between haplotype groups was confirmed by visual inspection of sequence alignments between individual accessions. This likely reflects high levels of gene conversion or recombination within the *OAK* gene.

A search of the Col-0 reference genome for the possible origin of the *OAK* promoter revealed that most of it probably arose from the coding region of one of the *RLK* genes, spanning intron 2 to exon 7 (encoding amino acids 207 to 383 of At5g59670). Although these regions are only 60 to 70% identical to the *OAK* promoter (BLASTN v2.2.25, E-value 1×10^{-61}), they present the best matches in the Col-0 genome (second best hit is to *LRR-RLK* gene At3g46330, E-value 3×10^{-13}) indicating that this is the most likely origin of the *OAK* promoter. While the promoter includes potential coding sequences, there are several in-frame stop codons upstream of the predicted OAK translation start. The $OAK_{\text{Bla-1}}$ and OAK_{Sha} promoters show similar levels of identity with *RLK* coding sequences across the cluster, but it seems most likely that the duplication of the At5g59670 gene involved an additional duplication that led to conversion of the region coding largely for the second malectin-like domain into a promoter. Interestingly, this is also the portion of the coding sequence that is most different between Bla-1 and Sha. The 260 bp promoter region immediately upstream of the start codon of *OAK* is most similar to sequences found in triplicate in the $\text{At5g59670}_{\text{Col-0}}$ promoter (Figure S9).

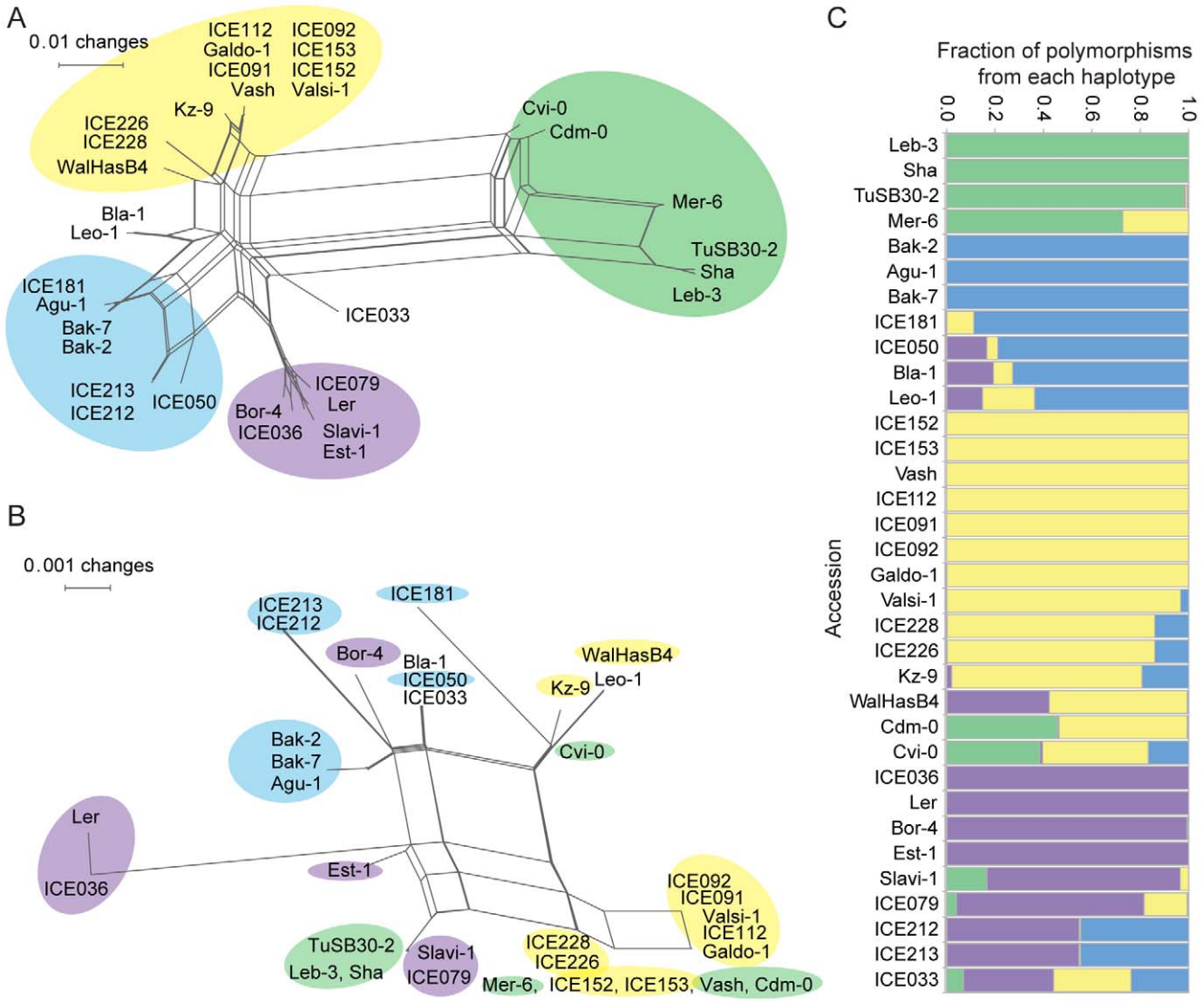


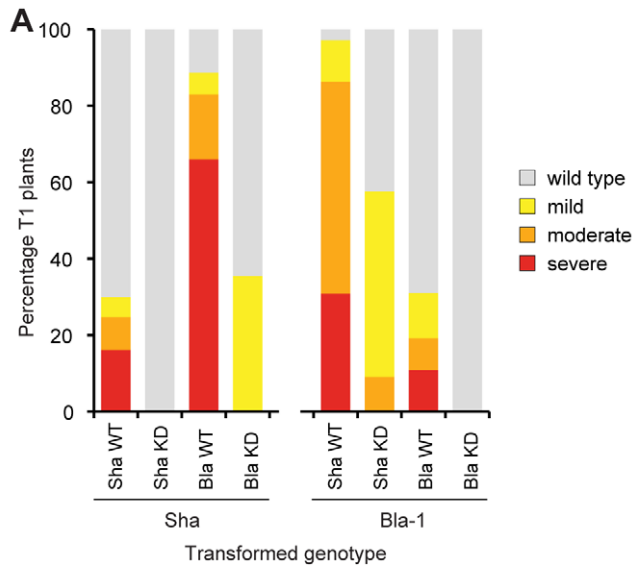
Figure 6. Phylogenetic analysis of *OAK* from multiple accessions. Splitstree [45] was used to examine the phylogenetic relationship of *OAK* from 34 accessions based on (a) 1,540 bp coding sequences downstream of the translational start site or (b) 1,196 bp promoter sequence. Color code in (b) reflects cluster membership in (a), highlighting variable correlation between promoter and coding region similarity. (c) STRUCTURE analysis [46] of haplotype contributions to each accession based on promoter and coding regions.
doi:10.1371/journal.pgen.1002164.g006

Role of the protein and kinase activity in causing the *OAK* hybrid phenotype

A second conclusion of the chimeric transgene experiments was that in addition to the promoter, the protein, and the extracellular domain in particular, contributed to the outgrowth phenotype (Figure 4a, 4b). The $At5g59670_{Col\text{-}0}$ protein did not cause an incompatibility phenotype even when expressed under the $OAK_{Bla\text{-}1}$ or OAK_{Sha} promoters. Swapping the extracellular and cytoplasmic domains between the $OAK_{Bla\text{-}1}$ and OAK_{Sha} proteins showed that the cytoplasmic domains were broadly equivalent. However, introduction of the extracellular domain of $OAK_{Bla\text{-}1}$ into the Sha genotype, or vice versa, greatly increased the proportion of affected T_1 plants. This result is supported by the incompatibility between Leo-1 and Sha, where Leo-1 has an extracellular domain identical to Bla-1, but only two amino acid differences in the cytoplasmic domain compared to Sha (Figure S10). Further attempts to narrow down the causal region within the extracellular domain with additional chimeras were not successful.

We tested the hypothesis that the outgrowth phenotype resulted from ectopic activation of a kinase-dependent signaling pathway by mutating key residues in the kinase catalytic domain [47]. Double mutants of D693N and K695R should lack all kinase activity. In the Sha background, over 80% of T_1 plants carrying the Bla-1 kinase-active construct had a moderate or severe phenotype, while only one third of T_1 plants transformed with the Bla-1 kinase-dead construct had any phenotype, and this was always mild. When the Sha kinase-dead construct was transformed back into the Sha accession, all T_1 transformants were wild type in appearance, which contrasts with 30% of T_1 plants expressing the Sha kinase-active construct having a mild to severe phenotype (Figure 7a). Results were comparable with Bla-1 transformants, although in this case some plants with a moderate phenotype were observed after transformation with the Sha kinase-dead construct.

Because RLKs can form homo- and heterodimers [48], we tested the effects of combining Bla-1 and Sha kinase-dead versions



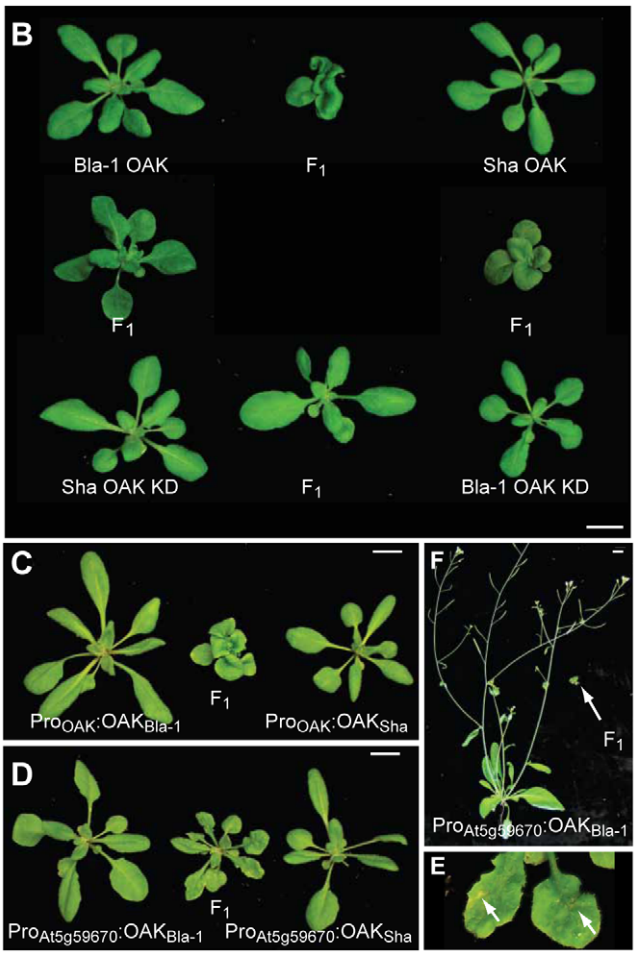


Figure 7. Requirement of *OAK* kinase activity and expression domain for hybrid phenotype. (a) Phenotypic distribution of T_1 plants ($n \geq 90$) expressing kinase dead (KD) or wild-type (WT) versions of OAK. (b) Crosses of Col-0 plants carrying Bla-1/Sha P_{OAK}:OAK KD constructs. Representative F_1 plants from crosses among five pairs of independent, phenotypically normal T_1 plants are shown with alongside the parental lines. Scale bar = 1 cm. (c) Crosses of five pairs of phenotypically normal Col-0 plants transformed with P_{OAK}:OAK_{Sha} and P_{OAK}:OAK_{Bla-1}, or (d, e) with $P_{At5g59670}$:OAK_{Sha} and $P_{At5g59670}$:OAK_{Bla-1}. Plants in (b-d) are 4-weeks old, in (e) 6-weeks old. Arrows in (f) indicate regions of cell death visible to the naked eye on a close-up of the F_1 plant in (d).
doi:10.1371/journal.pgen.1002164.g007

in the neutral Col-0 reference background. We transformed both kinase-active and -dead versions individually into Col-0 and then generated the four possible combinations by crossing (Figure 7b, 7c). The F_1 hybrids in which only one of the transgenes expressed a kinase-active version had a less severe phenotype than those carrying both Bla-1 and Sha kinase-active versions. All F_1 progeny from five crosses using OAK kinase-dead forms of both Bla-1 and Sha were wild type in appearance. This finding not only confirmed that kinase activity of OAK is required for its function, but also suggested that OAK can act as a heteroallelic dimer or multimer, because a kinase active version of one *OAK* allele can at least partially complement a kinase-dead version of the other *OAK* allele. In addition, these data indicated that other *RLKs* present at the *OAK* cluster in Col-0 are unlikely to be involved in the outgrowth phenotype.

Further circumstantial evidence suggesting that OAK proteins form dimers or multimers was obtained by expressing only the extracellular domain of OAK_{Bla-1} or OAK_{Sha} in hybrid plants. Expression under the native promoter in particular suppressed the outgrowth phenotype in many OAK_{Bla-1}/OAK_{Sha} heterozygous plants (Figure S11). We propose that by binding to OAK proteins, the extracellular domains reduce the number of active OAK_{Bla-1} or OAK_{Sha} heterodimers.

The OAK kinase can couple to the salicylic acid pathway

Curiosity led us to examine the consequences of mis-expressing the incompatible *OAK* alleles from the Col-0 promoter in the putative ancestral domain of the leaf lamina. We introduced $Pro_{At5g59670\text{-}Col}$:$OAK_{Bla}$ and $Pro_{At5g59670\text{-}Col}$:$OAK_{Sha}$ chimeric transgenes into the Col-0 reference background, and crossed the transformants, which were wild type in appearance, to each other. As described above, performing this experiment with the *OAK* wild-type alleles from Bla-1 and Sha reproduced the Bla-1/Sha hybrid phenotype with petiole outgrowths. Co-expressing the Bla-1 and Sha OAK proteins from the Col-0 promoter resulted in a new incompatibility phenotype, ranging from patches of cell death visible to the naked eye on the leaf lamina and abbreviated inflorescences, to severely stunted plants (Figure 7d–7f). It is striking that the altered expression domain leads essentially to a diametrically opposite phenotype, ectopic cell death instead of ectopic cell proliferation.

Tissue necrosis and ectopic cell death are typical responses to pathogen infection that rely on salicylic acid signaling [49]. To determine whether the cell death we observed was associated with increased activity of this pathway, we used a transgene that drives constitutive expression of a bacterial salicylate hydroxylase, nahG, which converts salicylic acid to catechol [50]. The Pro_{35S}:*nahG* transgene suppressed the cell death phenotype caused by co-expression of OAK_{Bla-1} and OAK_{Sha} proteins from the Col-0 promoter, but had no effect on the ectopic outgrowths and other phenotypes seen when the proteins were expressed from their own promoters in Col-0 (Figure S12). This not only indicated that OAK proteins can couple to alternative downstream signaling pathways (as is known for the BAK1 RLK [51]), but also that the ancestral function might have involved detection of microbes, a known function of different RLKs [52–54]. Mutation of other key genes in disease resistance pathways (*PAD4*, *EDS1*, and *NDR1*) [49] had no effect on the aberrant phenotypes caused by co-

expression of the OAK alleles under either the *OAK* or the Col-0 At5g59670 promoter.

Discussion

We have identified a case of a single-gene incompatibility interaction that leads to multiple aberrant phenotypes in hybrids between *A. thaliana* accessions Bla-1 and Sha. The phenotypes include reduced stature, leaf twisting, a loss of apical dominance and ectopic outgrowths on the petioles in addition to a decrease in lifetime fitness as measured by seed set. In the genetic sense, the Bla-1 and Sha OAK alleles can be thought of behaving in an overdominant fashion, since the action of either allele (which can cause milder versions of the hybrid phenotype in a foreign background on their own) is enhanced by the other allele. However, considering that the phenotypes are not normally seen in the parents or in other hybrids, and that one of them is reduced growth, the alleles behave in an underdominant fashion when it comes to fitness, as measured by seed set under laboratory conditions.

The causal gene for the Bla-1/Sha incompatibility, *OAK*, is an *RLK* that is part of a highly variable tandem array, with evidence of gene conversion, duplications and deletions in the recent evolutionary past. *OAK* was formed by a whole-gene duplication event in a common ancestor of Bla-1 and Sha, with the additional duplication of a segment of coding DNA that now forms most of the *OAK* promoter. This gene duplication is present in approximately one third of *A. thaliana* accessions sampled, but the Bla-1 and Sha alleles themselves are rare. The new promoter changed the *OAK* expression domain from the leaf lamina to the leaf petiole. Although this change is expression domain is required for manifestation of the *OAK* incompatibility, it is not in itself causal as the new promoter probably arose only once, and most accessions carrying the *OAK* gene are compatible with Bla-1 and Sha. Notably, the coding sequences that became part of the promoter include those coding for the second malectin-like domain, which has diverged between Bla-1, Sha and other accessions after the initial duplication. Changes in cis-regulatory sequences are an important source of interspecific variation [55], but such drastic intraspecific shifts in expression domains as we have observed are rare.

A function for OAK in disease resistance or development?

The *A. thaliana* genome encodes over 600 RLKs. Approximately two thirds of *A. thaliana* RLKs are predicted to contain structurally diverse extracellular domains [15], which often include LRRs [56]. These extracellular domains are involved in perceiving a wide range of ligands, including small proteins, steroids, and carbohydrates. The function and ligands of most plant RLKs are unknown, but known activities of LRR-RLKs include both control of plant development (e.g., BRI1 in brassinosteroid response [57], CLV1 in meristem maintenance [58] and ERECTA in pleiotropic patterning processes [59]) and microbe detection (e.g., Xa21, FLS2 and GmNARK [52–54]). The *RLK* genes constitute one of the most variable gene families in *A. thaliana*, which has been interpreted as many RLKs evolving in response to pathogen pressure [9]. Local and genome-wide duplications, along with gene conversion, have contributed to the expansion and diversification of *RLKs* in plants [12], and *RLK* genes are overrepresented in tandem arrays [15,60], although those with known roles in plant development are generally not located in tandem arrays [17].

Circumstantial evidence that might point to an interaction of OAK-like RLKs with microbes include the microarray results and the high variability of the *OAK* gene cluster. OAK does not appear to be required for normal development, since amiRNA-mediated knockdown of *OAK* activity has no obvious adverse effects. However, it is also possible that OAK acts redundantly in plant development given that the incompatibility phenotype manifests itself primarily as morphological abnormalities. In addition, the mis-expression experiments using the Col-0 promoter revealed that OAKs can trigger typical salicylic-acid dependent cell death as is often seen in response to pathogen attack, although OAK coupling to downstream signaling pathways may be dependent on the expression pattern of alternative interactors. Following the BAK1 paradigm [51], it is conceivable that the availability of OAK interaction partners determine its activity in plant development versus microbe-interactions. The similarity of the OAK extracellular domains to the carbohydrate-binding protein malectin [41] might indicate that OAK-like RLKs interact with carbohydrates found on the surface of microbes. Alternatively, their function might be detection of damaged self, according to the concept of indirect recognition of pathogens through damage-associated molecular patterns (DAMPs) [61]. A role for OAK in plant immunity through perception of self damage would be reminiscent of previously reported cases of hybrid incompatibility that involve disease resistance genes [6–8,62].

Causes for increased OAK activity in hybrids

Some RLKs function as hetero- or homodimers, with auto- and trans-phosphorylation required for function of the complex. For example, BAK1 and BRI1 form heteromultimers, and a multi-step pathway involving auto- and trans-phosphorylation events activates downstream signaling [63]. Our experiments with kinase-dead versions demonstrated that kinase activity is important for OAK function. The limited effects of the kinase-dead Sha allele in the Bla-1 background, and vice versa, indicate partial complementation by the opposite kinase-active allele, which is suggestive of heteroallelic dimer or multimer formation. In addition, the suppression of the hybrid phenotypes by expression of the Bla-1 or Sha OAK extracellular domain alone provides further support for this scenario.

We do not know whether the change in expression pattern associated with the acquisition of a new promoter by the Bla-1 and Sha *OAK* alleles subsequently became subject to positive selection, or whether these alleles lack a beneficial function all together. However, the fact that the unusually high divergence in sequence between the two alleles is largely restricted to the second malectin-like domains suggests positive selection or a gene conversion event. We speculate that these sequence changes also altered the affinity for potential ligands. The fact that the Bla-1 and Sha proteins on their own can cause a hybrid-like phenotype, albeit less effectively than when they are combined, suggests that each protein on its own can interact with this potential, unknown ligand. We speculate that OAK heterodimers have increased affinity for such a ligand, leading to ectopic activation of the downstream signaling pathway and aberrant development.

Evolution of incompatible *OAK* alleles

Several incompatibilities in F_1 and F_2 hybrids have recently been linked to disease resistance (*R*) genes. At least one of the *A. thaliana* factors, and likely another in *A. thaliana* and rice each, appears to be encoded in a highly polymorphic cluster of *NB-LRR* genes, the most common class of *R* genes, and at the same time the most polymorphic gene family in plants [6,8,9,62,64,65]. Indeed, more broadly, copy number variation is a recurring factor in reproductive isolation [66]. It has been proposed that the occurrence of disease resistance genes in clusters is critical for

generating diversity of resistance specificities, because the tandem arrays support high rates of gene conversion and illegitimate recombination [67]. Indeed, complex histories of transposon insertions, translocations, and gene duplications and rearrangements have also contributed to the formation of *NB-LRR* gene clusters [11,13,16,18,19]. *RLK* genes share with *NB-LRR* genes the frequent occurrence in tandem arrays and extreme diversity [9,12,15]. The complex evolutionary history of the *OAK* cluster is thus not atypical for this gene family.

Most hybrid incompatibilities described so far involve multiple loci and as such are classical examples of the Bateson, Dobzhansky and Muller model where derived alleles of two or more genes interact to produce underdominant fitness outcomes (e.g.[8,21, 62,68]). In contrast, the incompatibility we describe here is due to interaction of two different alleles at a single locus. Due to the high level of polymorphisms, it is difficult to know what the ancestral allele at the *OAK* locus looked like immediately after duplication. The incompatible *OAK* alleles may have evolved through mutations within both the Sha and Bla-1 lineages, with the current alleles remaining compatible with the ancestral allele. Alternatively, all important mutation and gene conversion events may have occurred in only one lineage, through multiple intermediate allelic forms that were never incompatible with the immediately ancestral allele [69]. Either way, evolution of the current situation would not require that plants passed through a fitness valley with heterozygosity for the two incompatible *OAK* alleles.

Conclusions

Not many cases of single-gene hybrid incompatibility have been described in plants: in rice, incompatible alleles of the *S5* locus cause most hybrids between the japonica and indica varieties to be female sterile [33]. It is not inconceivable that heterodimers are involved, similar to what appears to be the case for OAK, and dimer formation may be an important pre-condition for evolution of single-gene incompatibilities. We note that passage through a fitness valley is not required so long as the genetic changes causing incompatibility evolve in multiple steps within separate genetic backgrounds. In this way, two alleles could cause underdominance for fitness and reduce or abolish gene flow, but only upon crossing of lines that have diverged independently from a common ancestor. If there were strong positive selection for two different alleles that caused underdominance or sterility in hybrids, then they could eventually contribute to a speciation event.

In animals single-gene single-generation speciation occurs in snails, where shell chirality is maternally determined, with opposite chirality forming a strong pre-mating barrier [70,71]. Extenuating factors that could allow rapid speciation based on a single locus, even after one generation, include transient silencing of genes, for example, by parental imprinting, or incomplete sterility of the hybrid. If an incompatible allele arises, but is silenced for one generation, this would allow for the production of multiple offspring that are pre-or post-zygotically incompatible with individuals carrying the ancestral allele. Offspring with the new allele can self or interbreed to establish a subpopulation before this allele is lost again by genetic drift. Similarly, if the heteroallelic combination is sublethal, then F_2 offspring homozygous for the new allele can be produced. If, in turn, the homozygous form is subject to positive selection, the allele may become established in the population [70]. Such as scenario is particularly applicable to self-fertilizing species such as *Arabidopsis thaliana*.

Whether the sort of developmental abnormalities we have observed in Bla-1/Sha F_1 hybrids can contribute to cumulative reproductive isolation is of course not known. Nevertheless, that *OAK* has the potential to greatly reduce reproductive success can be inferred from the severe phenotypes in some plants transformed with active *OAK* constructs, the necrosis seen when incompatible OAKs are co-expressed from the Col-0 promoter, and the decrease in lifetime fitness as measured via seed set. All together, we propose that the occurrence of genes in variable tandem repeats, such as *NB-LRR* genes in several hybrid necrosis cases [6,8,62], or *RLKs* as in the present case, predisposes them to being sources for the creation of novel hybrid phenotypes. Whether, as with other mutations, these are normally disadvantageous or not, will require further systematic analyses of hybrid incompatibilities in a broad range of taxa.

Materials and Methods

Plant material

Bla-1 (N28079) and Sha (N28735) were obtained from the European Arabidopsis Stock Centre. Plants were grown at 16°C with 16 hours light, or 23°C with 8 or 16 hours of light, as indicated. Transgenic seedlings were selected on soil by BASTA resistance, and at least 90 T_1 plants phenotyped, unless otherwise indicated.

Transgenic plants

Genomic constructs spanned sequences from immediately downstream of the translational stop codon of the preceding gene to 200 bp downstream of the predicted translational stop. AmiRNAs were designed using WMD3 (http://wmd3.weigelworld.org/). Constructs were transformed into plants by the *Agrobacterium tumefaciens* floral-dip method [72] using strain GV3101 pMP90RK or ASE. For reporter gene analysis, the promoter region between the stop codon of the previous gene and the translational start codon of the *OAK* homolog was inserted into pGWB433 using Gateway LR clonase (Invitrogen, Darmstadt, Germany).

Seed set

Independent Pro_{OAK}:$OAK_{Bla\text{-}1}$ and Pro_{OAK}:OAK_{Sha} T_1 plants in Col-0 that did not show any morphological defects were crossed to each other to create F_1 populations, which were raised in randomly distributed individual pots without selection for the transgenes. Plants were genotyped, and seeds collected from each plant after three months of growth and weighed. The weight of individual seeds was determined by weighing 500 seeds for each of three plants per genotype, and total and individual seed weight were used to calculate total seed number per plant.

Humidity assay

Plants were grown in 23°C (long days) at 65% ambient humidity; or under mild drought-stress with minimal watering (but equal ambient humidity); or in saturated humidity with water surrounding the pots and the tray covered.

Histology

Bla-1 and Bla-1/Sha petioles were fixed in 3.7% formaldehyde, 5% acetic acid, 50% ethanol, embedded in an ASP300 (Leica, Nussloch, Germany) tissue processor in paraffin. Transverse sections of 8 µm thickness, stained with 0.02% Toluidine Blue after dewaxing, were examined with a Zeiss Axioplan 2 microscope.

Callus assay

Seeds were stratified for one week on ½ strength MS plates. Seedlings were grown in Percival LE Intellus chambers (Perry, IA,

USA) under 23°C long days until the 4-6 leaf stage. At least 40 transverse sections per genotype of leaves (1 mm thick) and petioles (2 mm thick) were placed on callus induction medium (3.1 g/L Gamborg's B5 salts, 2% glucose, 2.6 mM MES, pH 5.7, 0.8% agar) with 2.2 µM to 22 nM 2,4-dichlorophenoxyacetic acid (2,4-D) and 200 nM to 200 pM kinetin. Callus formation was assessed after 12 days.

Expression analysis

RNA was extracted from leaves of individual plants using the Qiagen (Hilden, Germany) Plant RNeasy Mini kit. One µg of RNA was DNaseI treated, and cDNA synthesized with hexamer primers (Fermentas RevertAid kit, St. Leon-Rot, Germany). qRT-PCR was performed with Invitrogen (St. Louis, MO, USA) SYBR Green PCR Mastermix and the MJR Opticon Continuous Fluorescence Detection System (Bio-Rad, Hercules, CA, USA). Two technical replicates were performed per sample. Expression was normalized to *β-TUBULIN-2* (At5g62690) and an amplification efficiency of 2.0 per cycle was used in the calculations. The average across three biological replicates is shown with standard deviation. The 5′ untranslated regions of OAK were identified by 5′ RACE (GeneRacer, Invitrogen, Darmstadt, Germany) on RNA from petioles (Bla-1 and Sha) or pedicels and peduncles (Sha).

GUS staining

Twelve-day old seedlings grown on ½ strength MS plates with kanamycin selection were fixed in 90% acetone on ice for 20 minutes. X-gluc stained tissue [72] was examined with a Leica MZFLIII microscope.

Microarrays

Affymetrix (Santa Clara, CA, USA) ATH1 microarrays were probed as described [73].

Genetic mapping

Coarse mapping was performed with the Sequenom (San Diego, CA, USA) MassARRAY platform. For high-resolution mapping, approximately 750 F_2 and F_3 plants were genotyped with microsatellite and CAPS markers [72].

Phylogenetic and statistical analyses

For the sliding window analysis of divergence, amino acid sequences were aligned with MUSCLE (http://www.ebi.ac.uk/Tools/muscle/) and nucleotide sequences with BlastX (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

For analysis of population structure, nucleotide sequences were aligned with Lasergene SeqMan. Networks were calculated with SplitsTree [45] using the default parameter settings for Neighbor-Net. For analysis of haplotypes and recombination, STRUCTURE (version 2.3.2.1) [46] was used with 200,000 iterations for the burnin and 800,000 iterations for the final analysis. A k value of 4 was used based on the SplitsTree results, with all other parameters as default.

Analyses of potential positive selection was performed with the *Codeml* programme implemented in PAML (version 3.15), using default settings [74]. A likelihood ratio test was used to identify residues under positive selection with Bayesian posterior probability calculated through the Bayes Empirical Bayes (BEB) tool [44]. Sites with $dN/dS>1$ and a high probability (>95%) are likely to be under positive selection.

A 2-way ANOVA analysis for interaction of lesioning, outgrowth formation and biomass was performed using a web service (http://faculty.vassar.edu/lowry/anova2×2.html).

Supporting Information

Figure S1 Bla-1/Sha incompatibility decreases seed set. (a) Normal appearing Col-0 plants that are either non-transgenic or carry only a single *OAK* transgene. The phenotype of F_1 plants with both *OAK* transgenes is comparable to (b) Sha/Bla-1 F_1 plants. (c) Total seed set after three months shown as box and whisker plots. Boxes cover the first and third quartile, and the whiskers represent values that are not more than 1.5 times the interquartile range. A two-tailed, unequal variance t-test showed statistical equivalence of seed set between wild-type plants and those with a single *OAK* transgene, and highly significant reduction of seed set in plants carrying both transgenes.
(TIF)

Figure S2 High humidity suppresses outgrowth formation. Bla-1/Sha F_1 plants were grown for 3 and a half weeks under either high humidity (covered with a dome and surrounded by water), normal humidity (controlled 65% humidity), or under drought stress conditions (65% humidity but minimal watering). Two representative leaves per treatment are shown. Outgrowths are indicated by arrows.
(TIF)

Figure S3 Effect of auxin and cytokinin concentration on callus formation. Callus formation at 12 days for transverse sections of leaves and petioles of Bla-1, Bla-1/Sha F1 and Sha. Three representative tissue pieces are shown per accession and hormone concentration.
(TIF)

Figure S4 Mapping interval for the Bla-1/Sha outgrowth causal gene. (a) Positional cloning markers used according to the cognate genes and position in Mbp in reference accession Col-0. (b) The genes in reference accession Col-0 in the final mapping interval, with protein kinases marked in light grey and the RLKs highlighted in mid-grey.
(TIF)

Figure S5 AmiRNA knockdown of *OAK* rescues the hybrid phenotype. AmiRNAs designed against each RLK in the *OAK* cluster from Bla-1 (a) or Sha (b) were transformed into Bla-1/Sha F_1 plants and plants heterozygous at the RLK locus identified in the next generation. One representative plant per line is shown. Scale bar = 1 cm.
(TIF)

Figure S6 Potential LRR and malectin-like domains in OAK. (a) The consensus for plant-specific LRR domains is given below according to (Kobe, B. & Kajava, A.V. The leucine-rich repeat as a protein recognition motif. *Curr. Opin. Struct. Biol.* 11, 725-32; 2001), with residues conserved in over 50% of proteins shown in uppercase. Leucine resides from OAK at conserved positions are indicated in yellow, with other conserved residues highlighted in green. Less conserved residues or residues similar to those conserved are highlighted in light grey. (b) Predicted malectin-like domains (Schallus, T. *et al.* Malectin: a novel carbohydrate-binding protein of the endoplasmic reticulum and a candidate player in the early steps of protein N-glycosylation. *Mol. Biol. Cell* 19, 3404-14; 2008) in OAK_{Bla-1} and OAK_{Sha}. Although the amino acid sequence identity is low (11–15%), the secondary structure is more highly conserved, and the probability scores are very high.
(DOC)

Figure S7 Divergence of RLK orthologs and paralogs. (a) Comparison of pairwise amino acid divergence between OAK_{Bla-1} and OAK_{Sha} and between all RLKs in this cluster. (b) Comparison

of pairwise amino acid divergence between OAK and At5g59670a alleles from Bla-1 and Sha.
(TIF)

Figure S8 Compatibility between *OAK*-containing accessions. Cytoscape (Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, *et al.* (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13: 2498–2504) representation of crosses performed between *OAK*-containing accessions (names indicated in circles). Node color on the periphery indicates the haplotype group of the second malectin domain. Cvi-0, Cdm-0, ICE50, ICE226 and ICE228 alleles switch between haplotype groups within the second malectin domain, and are shown in intermediate colors. Absence of color indicates that the haplotype group is not known. Compatible hybrid combinations are indicated by grey edges, while incompatible interactions with outgrowths are represented by black (hybrid phenotype of intensity similar to Sha/Bla-1), red (phenotypic onset early as for Sha/Bla-1 but milder leaf twisting and loss of apical dominance) or blue (late onset of outgrowths with no other incompatible phenotypes) edges.
(TIF)

Figure S9 Much of the *OAK* promoter is derived from a duplicated region of *RLK* coding sequence. Top 15 hits from LALIGN (http://www.ch.embnet.org/software/LALIGN_form.html) are shown according to position in the Bla-1 *OAK* promoter, linked to a color-matched box indicating position in the Col-0 *RLK* cluster.
(TIF)

Figure S10 Alignment of the OAK proteins from Sha, Leo-1 and Bla-1. Amino acid differences between the three OAK proteins are indicated in purple (where Sha differs from Leo-1 and Bla, which are both incompatible with Sha), in cyan (where Bla-1 differs from Sha and Leo-1) and in red (where Leo-1 differs from Sha and Bla-1). Alignment was performed with CLUSTALW (Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, *et al.* (2003) Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res 31: 3497–3500).
(TIF)

Figure S11 Expression of the OAK extracellular domain in hybrid plants can reduce the severity of aberrant phenotypes. The extracellular domains of OAK_{Sha}, OAK_{Bla} or $At5g59670_{Col\text{-}0}$ under control of their native promoters or the 35S promoter were transformed into a segregating hybrid background and scored for the hybrid phenotype. Transformants were genotyped for allelic status at the endogenous *OAK* locus to identify heterozygous individuals. Plants with a mild phenotype where only a few outgrowths were observed on the petioles but that were otherwise phenotypically wild-type were combined with the "wild-type" category.
(TIF)

Figure S12 Mis-expressed *OAK* couples to the salicylic acid signalling pathway. (a) Pro_{35S}*:nahG* when introduced into $P_{At5g59670}$*:*$OAK_{Bla\text{-}1}$ $P_{At5g59670}$*:*OAK_{Sha} rescues the cell death phenotype. (b) Pro_{35S}*:nahG* when introduced into P_{OAK}*:*$OAK_{Bla\text{-}1}$ P_{OAK}*:*OAK_{Sha} does not suppress the outgrowths, leaf twisting or loss of apical dominance.
(TIF)

Table S1 Outgrowth formation in short-day grown Bla-1 and Bla-1/Sha F_1 hybrids. Plants grown in 23°C short-day conditions were scored regularly for extopic outgrowths on the petioles.
(DOC)

Table S2 Outgrowth and lesioning phenotypes are correlated with reduced vegetative biomass. Average fresh weightof segregating sibling F_2 plants grown at 16°C for 5 weeks is reported.
(DOC)

Table S3 Overrepresented GO categories as determined by AmiGO among genes up- or down-regulated in Bla-1/Sha F_1 hybrids.
(DOC)

Table S4 Top ten up- and down-regulated genes in Bla-1/Sha F_1 hybrids compared to parental genotypes. See Table S5 for more information.
(DOC)

Table S5 Differentially regulated genes in Bla-1/Sha F_1 hybrids compared to parental genotypes.
(DOC)

Table S6 Similarity of *OAK* and related alleles. Nucleotide identity in percent is given on top, with amino acid identity given on bottom.
(DOC)

Table S7 Survey of 87 *A. thaliana* accessions for *OAK* duplication.
(DOC)

Acknowledgments

We thank Michael Hothorn (Salk Institute) for protein domain predictions; Eunyoung Chae for the identification of the Bla-1/Kond incompatibility; Eunyoung and Carmen Martin Pizarro for access to their many crosses; Yalong Guo for the *A. lyrata* sequences and population genetic analyses; Sang-tae Kim for assistance with STRUCTURE analysis; Stephan Ossowski for assistance with WMD3; and Richard Clark, John Willis, and Dani Zamir for critical reading of the manuscript.

Author Contributions

Conceived and designed the experiments: LMS DW KB. Performed the experiments: LMS KB. Analyzed the data: LMS. Wrote the paper: LMS DW.

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Supplementary Online Material for

Smith et al., Complex evolutionary events at a tandem cluster of *Arabidopsis thaliana* genes resulting in a single-locus genetic incompatibility

Supplementary Tables

Supplementary Table 1. Outgrowth formation in short-day grown Bla-1 and Bla-1/Sha F_1 hybrids.

Genotype	Experiment	*n*	Plants with outgrowths (%)	First leaf with outgrowths
Bla-1	1	40	65	24.1 ± 2.5
Bla-1	2	28	0	n/a
Bla-1/Sha F_1	1	39	100	11.8 ± 1.8

Plants grown in 23ºC short-day conditions were scored regularly for extopic outgrowths on the petioles.

Supplementary Table 2. Outgrowth and lesioning phenotypes are correlated with reduced vegetative biomass

	Weight* (± standard deviation)	
	Without outgrowths (*n*)	With outgrowths (*n*)
Not lesioned	1.58 ± 0.53 g (*27*)	1.12 ± 0.44 g (*39*)
Lesioned	0.66 ± 0.26 g (*16*)	0.74 ± 0.29 g (*32*)

*average fresh weight of segregating sibling F_2 plants grown at 16ºC for 5 weeks is reported.

Supplementary Table 3. Overrepresented GO categories as determined by AmiGO among genes up- or down-regulated in Bla-1/Sha F_1 hybrids.

Up-regulated genes	
GO category	**Enrichment p-value**
response to other organism	9.35×10^{-5}
response to stimulus	4.74×10^{-5}
response to biological stimulus	3.13×10^{-5}
response to jasmonic acid stimulus	6.20×10^{-4}
response to salicylic acid stimulus	4.70×10^{-3}
multi-organism processes	1.88×10^{-4}
catalytic activity	1.29×10^{-5}
Down-regulated genes	
GO category	**Enrichment p-value**
external encapsulating structure	7.37×10^{-3}
cell part	9.93×10^{-3}
catalytic activity	1.45×10^{-4}

Supplementary Table 4. Top ten up- and down-regulated genes in Bla-1/Sha F_1 hybrids compared to parental genotypes. See Supplementary Table 7 for more information.

Avg. fold change[a]		
		Up-regulated genes
6.3	AT1G13470	Unknown protein
6.2*	AT1G14870/AT1G14880	Uncharacterized protein
4.0	AT1G56140/AT1G56130/ AT1G56120	Leucine-rich repeat protein kinases
3.9	AT3G28290/AT3G28300	AT14A's, sequence similarity to integrins
3.8	AT3G48640	Unknown protein
3.6	AT2G18660	EXLB3 (EXPANSIN-LIKE B3 PRECURSOR)
3.5	AT4G23220	protein kinase family protein
3.3	AT1G22590	AGL87; transcription factor
3.2	AT5G54610	ANK (ANKYRIN); protein binding
3.2*	AT5G55450	proteinase inhibitor/seed storage lipid transfer protein (LTP) family protein
		Down-regulated genes
42.9	AT1G72910/AT1G72930	putative disease resistance proteins (TIR-NBS class)
26.3*	AT1G31580	ECS1
19.3	AT4G02850	phenazine biosynthesis PhzC/PhzF family protein
15.8	AT4G05050	UBQ11 (UBIQUITIN 11); protein binding
12.9	AT1G66690/AT1G66700	S-adenosyl-L-methionine:carboxyl methyltransferase family protein (AT1G66690); PXMT1; S-adenosylmethionine-dependent methyltransferase (AT1G66700)
12.7	AT4G29200	beta-galactosidase
11.6		
11.1	AT3G44430	Unknown protein
9.0	AT2G01090	ubiquinol-cytochrome C reductase complex 7.8 kDa protein, putative / mitochondrial hinge protein, putative
7.6	AT1G48598/AT1G48600	CPuORF31 (Conserved peptide upstream open reading frame 31) (AT1G48598); phosphoethanolamine N-methyltransferase 2, putative (NMT2) (AT1G48600)

[a]The smaller 'fold change' between the parent and hybrid is reported when there was no significant difference between the parental lines. In the remaining cases, indicated with an asterisk, the change relative to the average of the parents is given.

Supplementary Table 5. Differentially regulated genes in Bla-1/Sha F_1 hybrids compared to parental genotypes.

Down-regulated genes						
inverse FC	Average FC	Average pfp	Average P value	Array Element	Locus Identifier	Annotation
26.31578947	0.038	0	0	256497_at	AT1G31580	ECS1
4.33557338	0.23065	0.00595	0.00005	257365_x_at	AT2G26020	PDF1.2b (plant defensin 1.2b)
3.74181478	0.26725	0.0057	0.00005	249052_at	AT5G44420	PDF1.2 (Low-molecular-weight cysteine-rich 77)
3.702332469	0.2701	0.0004	0	255852_at	AT1G66970	glycerophosphoryl diester phosphodiesterase family protein
3.220611916	0.3105	0.00095	0	249942_at	AT5G22300	NIT4 (NITRILASE 4)
3.195398626	0.31295	0.01865	0.00015	258277_at	AT3G26830	PAD3 (PHYTOALEXIN DEFICIENT 3); oxygen binding
2.988196623	0.33465	0.00175	0	263046_at	AT2G05380	GRP3S (GLYCINE-RICH PROTEIN 3 SHORT ISOFORM)
2.754062242	0.3631	0.0022	0	266275_at	AT2G29370	tropinone reductase, putative / tropine dehydrogenase, putative
2.595380223	0.3853	0.0028	0	252698_at	AT3G43670	copper amine oxidase, putative
2.565418163	0.3898	0.00245	0	248377_at	AT5G51720	similar to Os07g0467200 [Oryza sativa (japonica cultivar-group)] (GB:NP_001059590.1); similar to hypothetical protein OsI_025030 [Oryza sativa (indica cultivar-group)] (GB:EAZ03798.1); contains domain PTHR13680 (PTHR13680); contains domain PTHR13680:SF1 (PTHR13680:SF1)
2.551671345	0.3919	0.00335	0	246420_at	AT5G16870	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT3G03010.1); similar to unknown protein [Arabidopsis thaliana] (TAIR:AT3G03010.2); similar to hypothetical protein [Vitis vinifera] (GB:CAN83813.1); contains InterPro domain Peptidyl-tRNA hydrolase, PTH2 (InterPro:IPR002833)
2.509725185	0.39845	0.0029	0	260151_at	AT1G52910	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT3G15480.1); similar to unknown [Populus trichocarpa] (GB:ABK94458.1); contains InterPro domain Protein of unknown function DUF1218 (InterPro:IPR009606)
2.501876407	0.3997	0.00285	0	260693_at	AT1G32450	proton-dependent oligopeptide transport (POT) family protein
2.501876407	0.3997	0.00325	0	257880_at	AT3G16910	AAE7/ACN1 (ACYL-ACTIVATING ENZYME 7); AMP binding / acetate-CoA ligase
2.409058058	0.4151	0.00405	0	254835_s_at	AT4G12320; AT4G12310	[AT4G12320, CYP706A6 (cytochrome P450, family 706, subfamily A, polypeptide 6); oxygen binding];[AT4G12310, CYP706A5 (cytochrome P450, family 706, subfamily A, polypeptide 5); oxygen binding]
2.396357537	0.4173	0.00385	0	263883_at	AT2G21830	DC1 domain-containing protein
2.385496183	0.4192	0.0056	0	259331_at	AT3G03840	auxin-responsive protein, putative
2.362111728	0.42335	0.0043	0	245331_at	AT4G14410	basic helix-loop-helix (bHLH) family protein

2.355435167	0.42455	0.0059	0	259658_at	AT1G55370	carbohydrate binding / catalytic
2.334539512	0.42835	0.0046	0	264365_s_at	AT1G03220; AT1G03230	[AT1G03220, extracellular dermal glycoprotein, putative / EDGP, putative];[AT1G03230, extracellular dermal glycoprotein, putative / EDGP, putative]
2.303351376	0.43415	0.0078	0.00005	265646_at	AT2G27360	lipase, putative
2.23015165	0.4484	0.00495	0	255447_at	AT4G02790	GTP-binding family protein
2.208480565	0.4528	0.0054	0	259468_at	AT3G55490; AT1G19080	[AT3G55490, similar to TTN10 (TITAN 10) [Arabidopsis thaliana] (TAIR:AT1G19080.1); similar to hypothetical protein [Vitis vinifera] (GB:CAN64086.1); contains InterPro domain GINS complex, Psf3 component (InterPro:IPR010492)];[AT1G19080, TTN10 (TITAN 10)]
2.186748305	0.4573	0.00895	0.00005	265665_at	AT2G27420	cysteine proteinase, putative
2.175805048	0.4596	0.00595	0	259238_at	AT3G11400	EIF3G1 (eukaryotic translation initiation factor 3G1); RNA binding / translation initiation factor
2.157497303	0.4635	0.01375	0.0002	254150_at	AT4G24350	phosphorylase family protein
2.135155333	0.46835	0.01405	0.00015	248943_s_at	AT5G45490; AT5G45440	[AT5G45490, disease resistance protein-related];[AT5G45440, disease resistance protein-related]
2.123367661	0.47095	0.01045	0.00005	267472_at	AT2G02850	ARPN (PLANTACYANIN); copper ion binding
2.123367661	0.47095	0.00895	0.00005	246478_at	AT5G15980	pentatricopeptide (PPR) repeat-containing protein
2.121565715	0.47135	0.0068	0.00005	254911_at	AT4G11100	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT5G03060.1)
2.066542674	0.4839	0.00925	0.00005	251722_at	AT3G56200	amino acid transporter family protein
2.043527128	0.48935	0.01095	0.0001	259224_at	AT3G07800	thymidine kinase, putative
2.041858091	0.48975	0.0097	0.00005	259403_at	AT1G17745	PGDH (3-PHOSPHOGLYCERATE DEHYDROGENASE); phosphoglycerate dehydrogenase
2.031900843	0.49215	0.01605	0.0001	264774_at	AT1G22890	unknown protein
2.013085053	0.49675	0.009	0.00005	250304_at	AT5G12110	elongation factor 1B alpha-subunit 1 (eEF1Balpha1)
2.011263073	0.4972	0.01355	0.00005	254612_at	AT4G19100	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT5G52780.1); similar to hypothetical protein [Vitis vinifera] (GB:CAN79943.1)
2.006018054	0.4985	0.01155	0.0001	259629_at	AT1G56510	disease resistance protein (TIR-NBS-LRR class), putative
1.994614541	0.50135	0.01015	0.00005	245400_at	AT4G17040	ATP-dependent Clp protease proteolytic subunit, putative
1.986097319	0.5035	0.0123	0.00005	263973_at	AT2G42740	RPL16A (ribosomal protein large subunit 16A); structural constituent of ribosome
1.977066034	0.5058	0.0116	0.0001	255451_at	AT4G02860	catalytic
1.976479889	0.50595	0.01975	0.00015	260551_at	AT2G43510	ATTI1 (ARABIDOPSIS THALIANA TRYPSIN INHIBITOR PROTEIN 1)
1.964250638	0.5091	0.0217	0.00015	248954_at	AT5G45420	myb family transcription factor

1.960976566	0.50995	0.0104	0.00005	262496_at	AT1G21790	similar to unnamed protein product [Vitis vinifera] (GB:CAO61872.1); contains InterPro domain TRAM, LAG1 and CLN8 homology; (InterPro:IPR006634)
1.935171746	0.51675	0.01425	0.0001	256336_at	AT1G72030	GCN5-related N-acetyltransferase (GNAT) family protein
1.92289203	0.52005	0.01305	0.0001	263177_at	AT1G05540	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G30160.2); contains InterPro domain Protein of unknown function DUF295 (InterPro:IPR005174)
1.922522349	0.52015	0.0172	0.00015	255604_at	AT4G01080	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G01430.1); similar to unknown protein Cr17 [Brassica napus] (GB:AAX51387.1); contains InterPro domain Protein of unknown function DUF231, plant (InterPro:IPR004253)
1.922337562	0.5202	0.01455	0.0001	249580_at	AT5G37740	C2 domain-containing protein
1.921045049	0.52055	0.03085	0.0003	265253_at	AT2G02020	proton-dependent oligopeptide transport (POT) family protein
1.916075877	0.5219	0.02135	0.00015	247849_at	AT5G58130	RNA recognition motif (RRM)-containing protein
1.911314985	0.5232	0.01365	0.0001	257805_at	AT3G18830	ATPLT5 (POLYOL TRANSPORTER 5); D-ribose transmembrane transporter/ D-xylose transmembrane transporter/ carbohydrate transmembrane transporter/ galactose transmembrane transporter/ glucose transmembrane transporter/ glycerol transmembrane transporter/ hydr
1.902587519	0.5256	0.0134	0.0001	251299_at	AT3G61950	basic helix-loop-helix (bHLH) family protein
1.888752479	0.52945	0.01755	0.00015	256397_at	AT3G06110	MKP2; protein tyrosine/serine/threonine phosphatase
1.87899286	0.5322	0.0195	0.00015	250114_s_at	AT5G16370; AT5G16340	[AT5G16370, AMP-binding protein, putative];[AT5G16340, AMP-binding protein, putative]
1.868634962	0.53515	0.0187	0.00015	245253_at	AT4G15440	HPL1 (HYDROPEROXIDE LYASE 1); heme binding / iron ion binding / monooxygenase
1.865845695	0.53595	0.0193	0.00015	267078_at	AT2G40960	nucleic acid binding
1.863759202	0.53655	0.01675	0.00015	259761_at	AT1G77590	LACS9 (LONG CHAIN ACYL-COA SYNTHETASE 9); long-chain-fatty-acid-CoA ligase
1.847404397	0.5413	0.0342	0.00035	252444_at	AT3G46980	transporter-related
1.844507977	0.54215	0.019	0.00015	258965_at	AT3G10530	transducin family protein / WD-40 repeat family protein
1.843487879	0.54245	0.02475	0.00035	258082_at	AT3G25905	CLE27 (CLAVATA3/ESR-RELATED 27); receptor binding
1.842638659	0.5427	0.02	0.00025	260653_at	AT1G32440	PKP3 (PLASTIDIAL PYRUVATE KINASE 3); pyruvate kinase
1.842638659	0.5427	0.024	0.0002	251165_at	AT3G63330	protein kinase family protein
1.839418744	0.54365	0.02115	0.00015	248763_at	AT5G47550	cysteine protease inhibitor, putative / cystatin, putative
1.821825469	0.5489	0.02275	0.0002	251024_at	AT5G02180	amino acid transporter family protein

1.820167455	0.5494	0.02115	0.0002	260453_s_at	AT1G72510; AT2G09970	[AT1G72510, similar to unknown protein [Arabidopsis thaliana] (TAIR:AT2G09970.1); similar to hypothetical protein [Vitis vinifera] (GB:CAN73516.1); contains InterPro domain Protein of unknown function DUF1677, plant (InterPro:IPR012876)];[AT2G09970, similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G72510.1); similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G72510.2); similar to hypothetical protein [Vitis vinifera] (GB:CAN73516.1); contains InterPro domain Protein of unknown function DUF1677, plant (InterPro:IPR012876)]
1.819505095	0.5496	0.0199	0.00025	248082_at	AT5G55400	fimbrin-like protein, putative
1.814058957	0.55125	0.0301	0.00035	263275_at	AT2G14170	ALDH6B2 (Aldehyde dehydrogenase 6B2); 3-chloroallyl aldehyde dehydrogenase
1.812415043	0.55175	0.03485	0.0004	245285_s_at	AT4G14030; AT4G14040	[AT4G14030, selenium-binding protein, putative];[AT4G14040, EDA38 (embryo sac development arrest 38); selenium binding]
1.807337791	0.5533	0.02815	0.00025	246966_at	AT5G24850	CRY3 (CRYPTOCHROME 3); DNA binding / DNA photolyase/ FMN binding
1.804891255	0.55405	0.0217	0.0002	265139_at	AT1G51310	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase
1.804402743	0.5542	0.02225	0.0002	249521_at	AT5G38690	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G67780.1); similar to unnamed protein product [Vitis vinifera] (GB:CAO49292.1); contains InterPro domain DDT (InterPro:IPR004022)
1.803589142	0.55445	0.02265	0.0003	245304_at	AT4G15630	integral membrane family protein
1.79937022	0.55575	0.0194	0.0002	259121_at	AT3G02220	similar to hypothetical protein [Cleome spinosa] (GB:ABD96929.1); contains domain PTHR22876:SF1 (PTHR22876:SF1); contains domain PTHR22876 (PTHR22876)
1.796299623	0.5567	0.02275	0.0002	254431_at	AT4G20840	FAD-binding domain-containing protein
1.791954126	0.55805	0.04535	0.00055	266038_at	AT2G07680	ATMRP11 (Arabidopsis thaliana multidrug resistance-associated protein 11)
1.791312136	0.55825	0.0252	0.00025	249372_at	AT5G40760	G6PD6 (GLUCOSE-6-PHOSPHATE DEHYDROGENASE 6); glucose-6-phosphate dehydrogenase

1.791151711	0.5583	0.02915	0.0003	248631_at	AT5G49000	Identical to F-box/Kelch-repeat protein At5g49000 [Arabidopsis Thaliana] (GB:Q9FI70;GB:Q8GY04); similar to kelch repeat-containing F-box family protein [Arabidopsis thaliana] (TAIR:AT4G39550.1); similar to 117M18_27 [Brassica rapa] (GB:AAZ66946.1); contains InterPro domain Kelch repeat type 1 (InterPro:IPR006652); contains InterPro domain Kelch-type beta propeller (InterPro:IPR015915); contains InterPro domain Cyclin-like F-box (InterPro:IPR001810); contains InterPro domain Kelch related (InterPro:IPR013089); contains InterPro domain Galactose oxidase/kelch, beta-propeller (InterPro:IPR011043)
1.787629603	0.5594	0.0211	0.00025	263902_at	AT2G36230	APG10 (ALBINO AND PALE GREEN 10); 1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4-carboxamide isomerase
1.776041204	0.56305	0.02495	0.00025	260603_at	AT1G55960	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT3G13062.2); similar to unknown protein [Arabidopsis thaliana] (TAIR:AT3G13062.1); similar to unnamed protein product [Vitis vinifera] (GB:CAO41766.1); contains InterPro domain Lipid-binding START (InterPro:IPR002913)
1.773521327	0.56385	0.0447	0.0005	254125_at	AT4G24670	alliinase family protein
1.770381517	0.56485	0.02665	0.00025	261016_at	AT1G26560	glycosyl hydrolase family 1 protein
1.770224819	0.5649	0.0434	0.0005	259807_at	AT1G47920	syntaxin-related family protein
1.76709666	0.5659	0.026	0.00025	250073_at	AT5G17170	ENH1 (ENHANCER OF SOS3-1); metal ion binding
1.763512918	0.56705	0.02605	0.00025	251995_at	AT3G52940	FK (FACKEL); delta14-sterol reductase
1.758550954	0.56865	0.0477	0.0006	247814_at	AT5G58310	hydrolase, alpha/beta fold family protein
1.750393839	0.5713	0.03	0.0003	257038_at	AT3G19260	LAG1 HOMOLOG 2 (LONGEVITY ASSURANCE GENE1 HOMOLOG 2)
1.737619461	0.5755	0.0338	0.00035	265025_at	AT1G24575	unknown protein
1.734454948	0.57655	0.0479	0.0008	250533_at	AT5G08640	FLS (FLAVONOL SYNTHASE)
1.724583944	0.57985	0.0355	0.00045	262113_at	AT1G02820	late embryogenesis abundant 3 family protein / LEA3 family protein
1.722504522	0.58055	0.03355	0.0006	261386_at	AT1G05430	similar to unnamed protein product [Vitis vinifera] (GB:CAO71187.1)
1.720726146	0.58115	0.03015	0.0005	257271_at	AT3G28007	nodulin MtN3 family protein
1.71688557	0.58245	0.03075	0.0003	247999_at	AT5G56150	UBC30 (UBIQUITIN-CONJUGATING ENZYME 30); ubiquitin-protein ligase
1.713649216	0.58355	0.0478	0.00125	255302_at	AT4G04830	methionine sulfoxide reductase domain-containing protein / SeIR domain-containing protein
1.7067759	0.5859	0.0309	0.00035	246329_at	AT3G43610	tubulin binding
1.702562356	0.58735	0.0438	0.00055	253191_at	AT4G35350	XCP1 (XYLEM CYSTEINE PEPTIDASE 1); cysteine-type peptidase

1.7008249	0.58795	0.0378	0.00055	252157_at	AT3G50430	similar to Os07g0120700 [Oryza sativa (japonica cultivar-group)] (GB:NP_001058781.1); similar to unnamed protein product [Vitis vinifera] (GB:CAO17953.1)
1.698946653	0.5886	0.0333	0.0004	248957_at	AT5G45620	26S proteasome regulatory subunit, putative (RPN9)
1.69333672	0.59055	0.0445	0.0005	262937_at	AT1G79560	EMB1047/FTSH12 (EMBRYO DEFECTIVE 1047); ATP-dependent peptidase/ ATPase/ metallopeptidase
1.692763436	0.59075	0.032	0.00035	259308_at	AT3G05180	GDSL-motif lipase/hydrolase family protein
1.691904238	0.59105	0.0496	0.0006	253666_at	AT4G30270	MERI5B (MERISTEM-5); hydrolase, acting on glycosyl bonds / xyloglucan:xyloglucosyl transferase
1.690045631	0.5917	0.03805	0.0004	255787_at	AT2G33590	cinnamoyl-CoA reductase family
1.68847615	0.59225	0.0328	0.00035	265183_at	AT1G23750	DNA-binding protein-related
1.686625063	0.5929	0.03905	0.0004	249239_at	AT5G41990; AT5G41992	[AT5G41990, WNK8 (Arabidopsis WNK kinase 8); kinase];[AT5G41992, CPuORF58 (Conserved peptide upstream open reading frame 58)]
1.685914187	0.59315	0.0349	0.00045	253050_at	AT4G37450	AGP18 (Arabinogalactan protein 18)
1.685772084	0.5932	0.0445	0.0005	256548_at	AT3G14770	nodulin MtN3 family protein
1.681944328	0.59455	0.04005	0.00045	249718_at	AT5G35740	glycosyl hydrolase family protein 17
1.680248677	0.59515	0.0337	0.00045	247541_at	AT5G61660	glycine-rich protein
1.664585934	0.60075	0.03665	0.00055	257132_at	AT3G20230	50S ribosomal protein L18 family
1.663755095	0.60105	0.0582	0.0008	255802_s_at	AT4G10150; AT4G10160	[AT4G10150, zinc finger (C3HC4-type RING finger) family protein];[AT4G10160, zinc finger (C3HC4-type RING finger) family protein]
1.662095903	0.60165	0.0414	0.00055	248684_at	AT5G48485	DIR1 (DEFECTIVE IN INDUCED RESISTANCE 1); lipid binding
1.657275439	0.6034	0.0397	0.0005	246702_at	AT5G28050	cytidine/deoxycytidylate deaminase family protein
1.651800463	0.6054	0.05215	0.00065	262262_at	AT1G70782; AT1G70780	[AT1G70782, CPuORF28 (Conserved peptide upstream open reading frame 28)];[AT1G70780, similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G23150.1); similar to unnamed protein product [Vitis vinifera] (GB:CAO42314.1)]
1.647310765	0.60705	0.0486	0.0007	256654_at	AT3G18880	ribosomal protein S17 family protein
1.646903821	0.6072	0.04355	0.0005	264201_at	AT1G22630	heat shock protein binding / unfolded protein binding
1.645955065	0.60755	0.03935	0.0005	248975_at	AT5G45040	cytochrome c6 (ATC6)
1.641766541	0.6091	0.04185	0.00055	254163_s_at	AT4G24340; AT4G24350	[AT4G24340, phosphorylase family protein];[AT4G24350, phosphorylase family protein]
1.634921932	0.61165	0.0463	0.0008	255486_at	AT4G02600	ATMLO1/MLO1 (MILDEW RESISTANCE LOCUS O 1); calmodulin binding
1.630390479	0.61335	0.04905	0.00095	258719_at	AT3G09540	pectate lyase family protein
1.627604167	0.6144	0.04535	0.00065	255240_at	AT4G05530	short-chain dehydrogenase/reductase (SDR) family protein
1.625884074	0.61505	0.04855	0.00065	254466_at	AT4G20430	subtilase family protein

1.625223468	0.6153	0.0569	0.0008	255011_at	AT4G10040	CYTC-2 (CYTOCHROME C-2); electron carrier
1.624827362	0.61545	0.0598	0.0008	264449_at	AT1G27460	NPGR1 (NO POLLEN GERMINATION RELATED 1); calmodulin binding
1.619039909	0.61765	0.0495	0.0007	250536_at	AT5G08535	D111/G-patch domain-containing protein
1.612773163	0.62005	0.0677	0.00105	251248_at	AT3G62150	PGP21 (P-GLYCOPROTEIN 21); ATPase, coupled to transmembrane movement of substances
1.612253124	0.62025	0.0526	0.0007	245340_at	AT4G14420	lesion inducing protein-related
1.61108426	0.6207	0.06075	0.00085	261738_s_at	AT1G47813; AT1G47820	[AT1G47813, similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G47820.1); similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G47820.2)];[AT1G47820, similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G47813.1); similar to unnamed protein product [Vitis vinifera] (GB:CAO40107.1)]
1.605651895	0.6228	0.05265	0.00075	254578_at	AT4G19410	pectinacetylesterase, putative
1.603720632	0.62355	0.0475	0.00075	248461_s_at	AT2G47510; AT5G50950	[AT2G47510, FUM1 (FUMARASE 1); fumarate hydratase];[AT5G50950, fumarate hydratase, putative / fumarase, putative]
1.60307791	0.6238	0.06325	0.00155	263134_at	AT1G78570	RHM1/ROL1 (RHAMNOSE BIOSYNTHESIS1); UDP-L-rhamnose synthase/ UDP-glucose 4,6-dehydratase/ catalytic
1.60012801	0.62495	0.0567	0.00075	262171_at	AT1G74950	JAZ2/TIFY10B (JASMONATE-ZIM-DOMAIN PROTEIN 2)
1.598465473	0.6256	0.0553	0.0012	258410_at	AT3G16780	60S ribosomal protein L19 (RPL19B)
1.598465473	0.6256	0.05825	0.0008	249733_at	AT5G24400	EMB2024 (EMBRYO DEFECTIVE 2024); catalytic
1.596169194	0.6265	0.0522	0.0009	251586_at	AT3G58070	GIS (GLABROUS INFLORESCENCE STEMS); nucleic acid binding / transcription factor/ zinc ion binding
1.588562351	0.6295	0.0536	0.00085	248191_at	AT5G54130	calcium-binding EF hand family protein
1.579030475	0.6333	0.05865	0.00125	245333_at	AT4G14615	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G52825.1); similar to unnamed protein product [Vitis vinifera] (GB:CAO71274.1)
1.578531965	0.6335	0.06145	0.0009	249847_at	AT5G23210	SCPL34; serine carboxypeptidase
1.57790927	0.63375	0.0673	0.00105	262286_at	AT1G68585	metal ion binding
1.574679159	0.63505	0.05875	0.00095	264984_at	AT1G27000	bZIP family transcription factor
1.573687938	0.63545	0.07845	0.00125	261508_at	AT1G71730	similar to unknown [Brassica rapa] (GB:ABL97948.1)
1.563599406	0.63955	0.06245	0.00115	249807_at	AT5G23870	pectinacetylesterase family protein
1.561889887	0.64025	0.06185	0.00105	266735_at	AT2G46930	pectinacetylesterase, putative
1.557389815	0.6421	0.0787	0.00145	262253_s_at	AT1G53900; AT1G53880	[AT1G53900, GTP binding / translation initiation factor];[AT1G53880, GTP binding / translation initiation factor]
1.55557284	0.64285	0.06715	0.0014	256091_at	AT1G20693	HMGB2 (HIGH MOBILITY GROUP B 2); transcription factor
1.552433439	0.64415	0.07085	0.0011	252542_at	AT3G45770	oxidoreductase, zinc-binding dehydrogenase family protein

1.547987616	0.646	0.07165	0.00125	253041_at	AT4G37870	PCK1/PEPCK (PHOSPHOENOLPYRUVATE CARBOXYKINASE 1); ATP binding / phosphoenolpyruvate carboxykinase (ATP)
1.546072975	0.6468	0.06435	0.00125	257867_at	AT3G17780	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G48440.1); similar to unknown [Populus trichocarpa] (GB:ABK93075.1)
1.537751807	0.6503	0.07335	0.00125	249920_at	AT5G19260	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT3G06020.1); similar to hypothetical protein [Vitis vinifera] (GB:CAN75990.1)
1.53233221	0.6526	0.0765	0.00135	246954_at	AT5G04830	similar to unknown [Populus trichocarpa x Populus deltoides] (GB:ABK96633.1); contains domain SSF54427 (SSF54427)
1.529051988	0.654	0.07805	0.00145	249327_at	AT5G40890	ATCLC-A (CHLORIDE CHANNEL A); anion channel/ voltage-gated chloride channel
1.526484506	0.6551	0.0786	0.0014	245506_at	AT4G15700	glutaredoxin family protein
1.519295047	0.6582	0.08095	0.00145	254815_at	AT4G12420	SKU5 (skewed 5); copper ion binding
1.51779616	0.65885	0.07715	0.0014	246762_at	AT5G27620	CYCH;1 (CYCLIN H;1); cyclin-dependent protein kinase/ protein binding / protein kinase
1.516990291	0.6592	0.08055	0.00175	258104_at	AT3G23620	brix domain-containing protein
1.513775356	0.6606	0.08075	0.00155	258033_at	AT3G21250	ATMRP6 (Arabidopsis thaliana multidrug resistance-associated protein 6)
1.513202694	0.66085	0.07865	0.00145	256304_at	AT1G69523	UbiE/COQ5 methyltransferase family protein
1.511601542	0.66155	0.07745	0.0016	253287_at	AT4G34270	TIP41-like family protein
1.508523156	0.6629	0.08205	0.00155	260794_at	AT1G06210	VHS domain-containing protein / GAT domain-containing protein
1.491646778	0.6704	0.08915	0.0019	260153_at	AT1G52760	esterase/lipase/thioesterase family protein
1.491313101	0.67055	0.0934	0.00185	249120_at	AT5G43750	similar to unnamed protein product [Vitis vinifera] (GB:CAO71280.1)

Down-regulated genes where there was a significant expression level difference between parents. Lowest fold change is reported only. (For all genes in this case it was Sha).

inverse FC	FC	pfp	P.value	Array Element	Locus Identifier	Annotation
42.91845494	0.0233	0	0	262374_s_at	AT1G72910; AT1G72930	[AT1G72910, disease resistance protein (TIR-NBS class), putative];[AT1G72930, TIR (TOLL/INTERLEUKIN-1 RECEPTOR-LIKE); transmembrane receptor]
19.26782274	0.0519	0	0	255450_at	AT4G02850	phenazine biosynthesis PhzC/PhzF family protein
15.82278481	0.0632	0	0	255257_at	AT4G05050	UBQ11 (UBIQUITIN 11); protein binding
12.88659794	0.0776	0	0	256376_s_at	AT1G66690; AT1G66700	[AT1G66690, S-adenosyl-L-methionine:carboxyl methyltransferase family protein];[AT1G66700, PXMT1; S-adenosylmethionine-dependent methyltransferase]
12.65822785	0.079	0	0	253707_at	AT4G29200	beta-galactosidase
11.61440186	0.0861	0	0	252659_at	AT3G44430	unknown protein
11.0864745	0.0902	0	0	262206_at	AT2G01090	ubiquinol-cytochrome C reductase complex 7.8 kDa protein, putative / mitochondrial hinge protein, putative
9.04159132	0.1106	0	0	261309_at	AT1G48598; AT1G48600	[AT1G48598, CPuORF31 (Conserved peptide upstream open reading frame 31)];[AT1G48600, phosphoethanolamine N-methyltransferase 2, putative (NMT2)]
7.604562738	0.1315	0	0	255065_s_at	AT4G08870; AT4G08900	[AT4G08870, arginase, putative];[AT4G08900, arginase]
7.490636704	0.1335	0	0	245729_at	AT1G73490	RNA recognition motif (RRM)-containing protein
7.132667618	0.1402	0	0	263023_at	AT1G23960	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G23970.1); contains InterPro domain Protein of unknown function DUF626, Arabidopsis thaliana (InterPro:IPR006462)
6.447453256	0.1551	0	0	258027_at	AT3G19515	binding
5.737234653	0.1743	0	0	246417_at	AT5G16990	NADP-dependent oxidoreductase, putative
5.730659026	0.1745	0	0	248944_at	AT5G45500	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT5G45520.1); similar to unnamed protein product [Vitis vinifera] (GB:CAO43141.1); similar to Os01g0799000 [Oryza sativa (japonica cultivar-group)] (GB:NP_001044526.1); contains domain SSF52047 (SSF52047); contains domain G3DSA:3.80.10.10 (G3DSA:3.80.10.10)
5.420054201	0.1845	0	0	245032_at	AT2G26630	transposable element gene

Up-regulated genes					
Average FC	Average p	Average F	Array Element	Locus Identifie	Annotation
6.14745	0.00025	0	262832_s_at	AT1G14870;A	[AT1G14870, Identical to Uncharacterized protein At1g14870 [Arabidop Thaliana] (GB:Q9LQU4); similar to unknown protein [Arabidopsis thalia (TAIR:AT5G35525.1); similar to unnamed protein product [Vitis vinifera (GB:CAO42338.1); contains InterPro domain Aspartic acid and asparag hydroxylation site (InterPro:IPR000152); contains InterPro domain Prot unknown function Cys-rich (InterPro:IPR006461)];[AT1G14880, similar protein [Arabidopsis thaliana] (TAIR:AT1G14870.1); similar to unnamed product [Vitis vinifera] (GB:CAO42338.1); similar to unnamed protein pr vinifera] (GB:CAO42335.1); contains InterPro domain Protein of unkno Cys-rich (InterPro:IPR006461)]
3.5968	0.0011	0	266070_at	AT2G18660	EXLB3 (EXPANSIN-LIKE B3 PRECURSOR)
3.4526	0.00065	0	254255_at	AT4G23220	protein kinase family protein
3.15565	0.0033	0	248062_at	AT5G55450	protease inhibitor/seed storage/lipid transfer protein (LTP) family protei
3.06945	0.03985	0.00105	250445_at	AT5G10760	aspartyl protease family protein
2.96	0.00365	0	249096_at	AT5G43910	pfkB-type carbohydrate kinase family protein
2.9273	0.0038	0	245329_at	AT4G14365	zinc finger (C3HC4-type RING finger) family protein / ankyrin repeat far
2.92105	0.00235	0	265228_s_at	ATMG01190;A	[ATMG01190, ATPase subunit 1];[AT2G07698, ATP synthase alpha ch mitochondrial, putative]
2.83215	0.00175	0	248810_at	AT5G47280	ADR1-L3 (ADR1-LIKE 3); ATP binding / nucleoside-triphosphatase/ nu binding / protein binding
2.82005	0.00255	0	245422_at	AT4G17470	palmitoyl protein thioesterase family protein
2.7618	0.00265	0	247604_at	AT5G60950	COBL5 (COBRA-LIKE PROTEIN 5 PRECURSOR)
2.7256	0.0068	0.00005	254521_at	AT5G44820	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT4G19970.1); unnamed protein product [Vitis vinifera] (GB:CAO46707.1); contains do PTHR10483:SF6 (PTHR10483:SF6); contains domain PTHR10483 (PT
2.7202	0.0038	0	259561_at	AT1G21250	WAK1 (CELL WALL-ASSOCIATED KINASE); kinase
2.60305	0.03915	0.00105	251673_at	AT3G57240	BG3 (BETA-1,3-GLUCANASE 3); hydrolase, hydrolyzing O-glycosyl co
2.46155	0.0093	0.0001	253423_at	AT4G32280	IAA29 (indoleacetic acid-induced protein 29); transcription factor
2.4512	0.0039	0	250277_at	AT5G12940	leucine-rich repeat family protein
2.45005	0.0044	0	245265_at	AT4G14400	ACD6 (ACCELERATED CELL DEATH 6); protein binding
2.4438	0.00675	0.00005	259272_at	AT3G01290	band 7 family protein
2.4005	0.0159	0.00025	248327_at	AT5G52750	heavy-metal-associated domain-containing protein
2.35755	0.0059	0.00005	258856_at	AT3G02040	SRG3 (SENESCENCE-RELATED GENE 3); glycerophosphodiester phosphodiesterase
2.3575	0.00805	0.00005	265441_at	AT2G20870	cell wall protein precursor, putative
2.31875	0.01085	0.0001	249813_at	AT5G23940	EMB3009 (EMBRYO DEFECTIVE 3009); transferase

2.3185	0.00565	0.00005	248330_at	AT5G52810	ornithine cyclodeaminase/mu-crystallin family protein
2.2809	0.0097	0.0001	266643_s_at	AT2G29730;A-	[AT2G29730, UDP-glucoronosyl/UDP-glucosyl transferase family protein];[AT2G29710, UDP-glucoronosyl/UDP-glucosyl transferase fam
2.25645	0.007	0.00005	245076_at	AT2G23170	GH3.3; indole-3-acetic acid amido synthetase
2.24965	0.0109	0.00005	252184_at	AT3G50660	DWF4 (DWARF 4)
2.2399	0.02295	0.0002	251347_at	AT3G61010	glycosyl hydrolase family protein 85
2.22535	0.0141	0.0002	245052_at	AT2G26440	pectinesterase family protein
2.211	0.00815	0.00005	252213_at	AT3G50210	2-oxoacid-dependent oxidase, putative
2.1688	0.03445	0.0008	265067_at	AT1G03850	glutaredoxin family protein
2.13885	0.04765	0.0008	266423_at	AT2G41340	eukaryotic rpb5 RNA polymerase subunit family protein
2.1116	0.03045	0.00065	262926_s_at	AT1G65800;A-	[AT1G65800, ARK2 (Arabidopsis Receptor Kinase 2); kinase];[AT1G65 (A. THALIANA RECEPTOR KINASE I); kinase]
2.11145	0.0038	0	254521_at	AT5G44820	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT4G19970.1); unnamed protein product [Vitis vinifera] (GB:CAO46707.1); contains do
2.1091	0.01625	0.00015	254579_at	AT4G19400	PTHR10483:SF6 (PTHR10483:SF6); contains domain PTHR10483 (PT actin binding
2.1065	0.01445	0.0001	264843_at	AT1G03400	2-oxoglutarate-dependent dioxygenase, putative
2.09985	0.0169	0.00015	252414_at	AT3G47420	glycerol-3-phosphate transporter, putative / glycerol 3-phosphate perme putative
2.09265	0.01505	0.00015	262844_at	AT1G14890	pectinesterase inhibitor
2.08745	0.0127	0.0001	252365_at	AT3G48350	cysteine proteinase, putative
2.08105	0.01625	0.00015	254247_at	AT4G23260	protein kinase
2.0637	0.0258	0.0005	266993_at	AT2G39210	nodulin family protein
2.06305	0.013	0.0001	253493_at	AT4G31820	ENP (ENHANCER OF PINOID); signal transducer
2.03105	0.01475	0.00015	261969_at	AT1G65950	ABC1 family protein
2.0295	0.01225	0.0001	266613_at	AT2G14900	gibberellin-regulated family protein
2.0279	0.03185	0.00035	261193_at	AT1G32920	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G32928.1)
2.0243	0.0213	0.00035	252976_s_at	AT4G38550	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT2G20950.1); InterPro domain Phospholipase-like, arabidopsis (InterPro:IPR007942)
2.01655	0.0428	0.00065	256863_at	AT3G24070	zinc knuckle (CCHC-type) family protein
2.01425	0.0187	0.00015	255931_at	AT1G12710	ATPP2-A12 (PHLOEM PROTEIN 2-A12); carbohydrate binding
2.0117	0.01755	0.00015	264838_at	AT1G03430	AHP5 (HISTIDINE-CONTAINING PHOSPHOTRANSFER FACTOR 5); phosphotransfer kinase
2.00845	0.0188	0.0002	260772_at	AT1G49050	aspartyl protease family protein
2.0058	0.01795	0.00015	252652_at	AT3G44720	ADT4 (AROGENATE DEHYDRATASE 4); arogenate dehydratase/ prep dehydratase
1.98075	0.01535	0.00015	267529_at	AT2G45490	ATAUR3 (ATAURORA3); ATP binding / histone serine kinase(H3-S10 s protein kinase

1.9806	0.0154	0.00015	258158_at	AT3G17790	ATACP5 (acid phosphatase 5); acid phosphatase/ protein serine/threor phosphatase
1.9697	0.01975	0.00015	252387_at	AT3G47800	aldose 1-epimerase family protein
1.96025	0.055	0.00095	265993_at	AT2G24160	pseudogene, leucine rich repeat protein family, contains leucine rich-re domains Pfam:PF00560, INTERPRO:IPR001611; contains some simila (Lycopersicon hirsutum) gi|2808683|emb|CAA05268; blastp match of 3 and 8.4e-98 P-value to GP|2808683|emb|CAA05268.1||AJ002235 Cf-4 {Lycopersicon hirsutum}
1.95875	0.02765	0.0003	247742_at	AT5G58980	ceramidase family protein
1.9569	0.01855	0.0002	250828_at	AT5G05250	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT3G56360.1); unnamed protein product [Vitis vinifera] (GB:CAO41488.1)
1.9555	0.0487	0.0013	263852_at	AT2G04450	ATNUDT6 (Arabidopsis thaliana Nudix hydrolase homolog 6); ADP-ribo diphosphatase/ NAD binding / hydrolase
1.95245	0.02355	0.0002	258379_at	AT3G16700	fumarylacetoacetate hydrolase family protein
1.9507	0.02675	0.00025	250931_at	AT5G03200	zinc finger (C3HC4-type RING finger) family protein
1.94855	0.0178	0.00015	262312_at	AT1G70830	MLP28 (MLP-LIKE PROTEIN 28)
1.9434	0.02865	0.0003	247419_at	AT5G63080	transcription factor jumonji (jmjC) domain-containing protein
1.9386	0.0183	0.00025	260567_at	AT2G43820	GT/UGT74F2 (UDP-GLUCOSYLTRANSFERASE 74F2); UDP-glucosyltransferase/ UDP-glycosyltransferase/ transferase, transferring groups / transferase, transferring hexosyl groups
1.938	0.021	0.00025	252117_at	AT3G51430	YLS2 (yellow-leaf-specific gene 2); strictosidine synthase
1.93185	0.02025	0.0002	260077_at	AT1G73620	thaumatin-like protein, putative / pathogenesis-related protein, putative
1.92305	0.02165	0.00025	249918_at	AT5G19240	Identical to Uncharacterized GPI-anchored protein At5g19240 precurso [Arabidopsis Thaliana] (GB:Q84VZ5;GB:Q8H7A4); similar to unknown [Arabidopsis thaliana] (TAIR:AT5G19230.1); similar to unknown [Popul trichocarpa] (GB:ABK94712.1)
1.91915	0.01975	0.00025	261032_at	AT1G17430	hydrolase, alpha/beta fold family protein
1.91375	0.02275	0.0002	254256_at	AT4G23180	CRK10 (CYSTEINE-RICH RLK10); kinase
1.89615	0.02385	0.00025	261071_at	AT1G07380	ceramidase family protein
1.89195	0.024	0.00035	244901_at	ATMG00640	encodes a plant b subunit of mitochondrial ATP synthase based on stru similarity and the presence in the F(0) complex.
1.88845	0.03055	0.00035	267432_at	AT2G35020	UTP--glucose-1-phosphate uridylyltransferase family protein
1.8884	0.02775	0.0003	257285_at	AT3G29760	NLI interacting factor (NIF) family protein
1.8856	0.0435	0.0006	247530_at	AT5G61540	L-asparaginase, putative / L-asparagine amidohydrolase, putative
1.88265	0.026	0.0003	254232_at	AT4G23600	CORI3 (CORONATINE INDUCED 1, JASMONIC ACID RESPONSIVE transaminase
1.8824	0.061	0.00105	252403_at	AT3G48080	lipase class 3 family protein / disease resistance protein-related
1.8822	0.0313	0.00035	258530_at	AT3G06840	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT5G49170.1); unnamed protein product [Vitis vinifera] (GB:CAO44815.1)

1.8812	0.05095	0.0008	251035_at	AT5G02220	similar to unknown [Picea sitchensis] (GB:ABK23883.1); similar to hypo protein [Vitis vinifera] (GB:CAN70860.1)
1.88	0.0242	0.0004	245399_at	AT4G17340	DELTA-TIP2/TIP2;2 (tonoplast intrinsic protein 2;2); water channel
1.87965	0.02975	0.0005	262888_at	AT1G14790	RDR1 (RNA-DEPENDENT RNA POLYMERASE 1); RNA-directed RNA polymerase/ nucleic acid binding
1.8731	0.0327	0.0004	251668_at	AT3G57010	strictosidine synthase family protein
1.8705	0.0466	0.00075	251705_at	AT3G56400	WRKY70 (WRKY DNA-binding protein 70); transcription factor
1.8668	0.0297	0.00055	253238_at	AT4G34480	glycosyl hydrolase family 17 protein
1.8657	0.02635	0.0004	251422_at	AT3G60540	sec61beta family protein
1.86385	0.0264	0.00035	253377_at	AT4G33300	ADR1-L1 (ADR1-LIKE 1); ATP binding / protein binding
1.8551	0.03015	0.00045	254283_s_at	AT4G22870;A	[AT4G22870, leucoanthocyanidin dioxygenase, putative / anthocyanidi putative];[AT4G22880, LDOX (TANNIN DEFICIENT SEED 4)]
1.85185	0.0253	0.00025	267096_at	AT2G38180	GDSL-motif lipase/hydrolase family protein
1.85065	0.0309	0.00035	262910_at	AT1G59710	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G27100.1); unknown [Populus trichocarpa] (GB:ABK94560.1); contains InterPro do Protein of unknown function DUF569 (InterPro:IPR007679); contains In domain Actin-crosslinking proteins (InterPro:IPR008999)
1.84555	0.02735	0.0003	263953_at	AT2G36050	ATOFP15/OFP15 (Arabidopsis thaliana ovate family protein 15)
1.83975	0.0263	0.0003	246071_at	AT5G20150	SPX (SYG1/Pho81/XPR1) domain-containing protein
1.83575	0.0303	0.00055	250937_at	AT5G03230	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT5G60680.1); unnamed protein product [Vitis vinifera] (GB:CAO21845.1); contains Int domain Protein of unknown function DUF584 (InterPro:IPR007608)
1.83555	0.03125	0.00035	258173_at	AT3G21630	CERK1 (CHITIN ELICITOR RECEPTOR KINASE 1); kinase/ receptor s protein/ transmembrane receptor protein kinase
1.82585	0.03365	0.0004	253401_at	AT4G32870	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT2G25770.2); unknown protein [Arabidopsis thaliana] (TAIR:AT2G25770.1); similar to [Populus trichocarpa x Populus deltoides] (GB:ABK96434.1); contains SSF55961 (SSF55961)
1.82375	0.0281	0.0005	250661_at	AT5G07030	pepsin A
1.81815	0.03315	0.00035	249904_at	AT5G22700	F-box family protein
1.81525	0.04665	0.0012	261240_at	AT1G32940	ATSBT3.5; subtilase
1.8127	0.04035	0.0005	253722_at	AT4G29190	zinc finger (CCCH-type) family protein
1.80975	0.02725	0.00035	245602_at	AT4G14270	Protein containing PAM2 motif which mediates interaction with the PAB of polyadenyl binding proteins.
1.8091	0.02905	0.00035	248248_at	AT5G53120	SPDS3 (SPERMIDINE SYNTHASE 3)
1.80425	0.0295	0.0004	258786_at	AT3G11820	SYP121 (syntaxin 121); SNAP receptor
1.8041	0.0536	0.00085	255294_at	AT4G04750	carbohydrate transmembrane transporter/ sugar:hydrogen ion symporte
1.80165	0.0488	0.0008	267246_at	AT2G30250	WRKY25 (WRKY DNA-binding protein 25); transcription factor
1.8007	0.0287	0.0004	267423_at	AT2G35060	KUP11 (K+ uptake permease 11); potassium ion transmembrane trans

1.7999	0.0433	0.0006	250891_at	AT5G04530	beta-ketoacyl-CoA synthase family protein
1.7995	0.051	0.00075	263914_at	AT2G36400	AtGRF3 (GROWTH-REGULATING FACTOR 3)
1.79825	0.0298	0.0005	247632_at	AT5G60460	sec61beta family protein
1.79375	0.04	0.0005	258351_at	AT3G17700	CNBT1 (CYCLIC NUCLEOTIDE-BINDING TRANSPORTER 1); calmoc / cyclic nucleotide binding / ion channel
1.78845	0.0444	0.0006	251010_at	AT5G02550	unknown protein
1.78635	0.05095	0.0008	259009_at	AT3G09260	PYK10 (phosphate starvation-response 3.1); hydrolase, hydrolyzing O- compounds
1.7859	0.0359	0.00055	250083_at	AT5G17220	ATGSTF12 (GLUTATHIONE S-TRANSFERASE 26); glutathione transf
1.78395	0.03345	0.00045	264787_at	AT2G17840	ERD7 (EARLY-RESPONSIVE TO DEHYDRATION 7)
1.7803	0.0395	0.0005	245074_at	AT2G23200	protein kinase family protein
1.7794	0.03065	0.00045	245302_at	AT4G17695	KAN3 (KANADI 3); DNA binding / transcription factor
1.7741	0.0312	0.0004	267595_at	AT2G32990	ATGH9B8 (ARABIDOPSIS THALIANA GLYCOSYL HYDROLASE 9B8 hydrolase, hydrolyzing O-glycosyl compounds
1.7735	0.03605	0.0006	264223_s_at	AT3G16030	CES101 (CALLUS EXPRESSION OF RBCS 101); carbohydrate bindin
1.7703	0.04765	0.00065	246905_at	AT5G25570	similar to unnamed protein product [Vitis vinifera] (GB:CAO44135.1)
1.7681	0.0434	0.0006	263582_at	AT2G17120	LYM2 (LYSM DOMAIN GPI-ANCHORED PROTEIN 2 PRECURSOR)
1.7665	0.0462	0.0007	251641_at	AT3G57470	peptidase M16 family protein / insulinase family protein
1.7632	0.03435	0.00045	266202_at	AT2G02400	cinnamoyl-CoA reductase family
1.76045	0.04095	0.00055	262455_at	AT1G11310	MLO2 (MILDEW RESISTANCE LOCUS O 2); calmodulin binding
1.7564	0.04825	0.0007	262736_at	AT1G28570	GDSL-motif lipase, putative
1.7461	0.0403	0.0006	248794_at	AT5G47220	ATERF-2/ATERF2/ERF2 (ETHYLENE RESPONSE FACTOR 2); DNA transcription activator/ transcription factor
1.74405	0.04065	0.0006	254909_at	AT4G11210	disease resistance-responsive family protein / dirigent family protein
1.7439	0.0563	0.0009	265142_at	AT1G51360	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT2G31670.1); unknown [Populus trichocarpa] (GB:ABK93857.1); contains InterPro do Dimeric alpha-beta barrel (InterPro:IPR011008); contains InterPro dom responsive alpha-beta barrel (InterPro:IPR013097)
1.74325	0.04375	0.00085	244951_s_at	AT2G07723;A	[AT2G07723, pseudogene, similar to orf454~homology with two ORFs Marchantia polymorpha mtDNA (orf169 and orf322), high similarity to 3 part of ccl1 of Rhodobacter, blastp match of 76% identity and 3.4e-193 GP|459537|emb|CAA54966.1||X78036 orf454~homology with two ORF Marchantia polymorpha mtDNA (orf169 and orf322), high similarity to 3 part of ccl1 of Rhodobacter {Oenothera berteriana}];[ATMG00180, cyto biogenesis orf452]
1.73875	0.04805	0.0007	247284_at	AT5G64410	ATOPT4 (oligopeptide transporter 4); oligopeptide transporter
1.73605	0.0416	0.0008	264854_at	AT2G17450	RHA3A (RING-H2 finger A3A); protein binding / zinc ion binding
1.73425	0.06015	0.001	248568_at	AT5G49760	leucine-rich repeat family protein / protein kinase family protein

1.7307	0.05485	0.00095	259990_s_at	AT5G23410;A- AT5G42730	[AT5G23410, similar to FKF1 (FLAVIN-BINDING KELCH DOMAIN F B PROTEIN), ubiquitin-protein ligase [Arabidopsis thaliana] (TAIR:AT1G6 similar to unnamed protein product [Vitis vinifera] (GB:CAO42365.1); co domain PTHR23244 (PTHR23244); contains domain PTHR23244:SF9 (PTHR23244:SF9)];[AT1G68050, FKF1 (FLAVIN-BINDING KELCH DO BOX PROTEIN); ubiquitin-protein ligase];[AT5G42730, pseudogene sir domain-containing protein, similar to F-box family protein]
1.7304	0.03795	0.0006	257377_at	AT2G28890	PLL4 (POLTERGEIST LIKE 4); protein serine/threonine phosphatase
1.7199	0.04505	0.0007	246897_at	AT5G25560	zinc finger (C3HC4-type RING finger) family protein
1.7193	0.0551	0.0012	247602_at	AT5G60900	RLK1 (RECEPTOR-LIKE PROTEIN KINASE 1); carbohydrate binding /
1.71885	0.0562	0.0013	263478_at	AT2G31880	leucine-rich repeat transmembrane protein kinase, putative
1.71815	0.04215	0.0006	253788_at	AT4G28680	tyrosine decarboxylase, putative
1.71345	0.0611	0.00105	267490_at	AT2G19130	S-locus lectin protein kinase family protein
1.71135	0.0414	0.0006	247735_at	AT5G59440	thymidylate kinase family protein
1.7086	0.051	0.0008	258196_at	AT3G13980	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G54200.1); hypothetical protein [Vitis vinifera] (GB:CAN69469.1)
1.69585	0.0526	0.00135	253753_at	AT4G29030	glycine-rich protein
1.69475	0.05495	0.00085	250810_at	AT5G05090	myb family transcription factor
1.6914	0.04695	0.001	256922_at	AT3G19010	oxidoreductase, 2OG-Fe(II) oxygenase family protein
1.6891	0.055	0.00085	262821_at	AT1G11800	endonuclease/exonuclease/phosphatase family protein
1.6883	0.05465	0.0009	254609_at	AT4G18970	GDSL-motif lipase/hydrolase family protein
1.684	0.05345	0.0012	266320_at	AT2G46640	unknown protein
1.6809	0.0456	0.0008	252560_at	AT3G46030	HTB11; DNA binding
1.6786	0.07375	0.00145	260046_at	AT1G73805	calmodulin binding
1.67825	0.0513	0.00115	263578_at	AT2G17020	F-box family protein (FBL10)
1.6782	0.0542	0.0009	265118_at	AT1G62660	beta-fructosidase (BFRUCT3) / beta-fructofuranosidase / invertase, vac
1.6743	0.0488	0.001	248912_at	AT5G45670	GDSL-motif lipase/hydrolase family protein
1.66415	0.0481	0.0009	247275_at	AT5G64370	BETA-UP (BETA-UREIDOPROPIONASE); beta-ureidopropionase
1.6614	0.05055	0.00115	257769_at	AT3G23050	IAA7 (AUXIN RESISTANT 2); transcription factor
1.65875	0.05185	0.0009	259165_at	AT3G01472;A-	[AT3G01472, CPuORF33 (Conserved peptide upstream open reading f 33)];[AT3G01470, ATHB-1 (Homeobox-leucine zipper protein HAT5); tr factor]
1.65815	0.06515	0.00115	253967_at	AT4G26550	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT5G56020.1); unnamed protein product [Vitis vinifera] (GB:CAO45187.1); contains Int domain SFT2-like (InterPro:IPR011691)
1.6561	0.0652	0.00115	254858_at	AT4G12070	protein binding
1.6553	0.0537	0.00095	255500_at	AT4G02390	APP (ARABIDOPSIS POLY(ADP-RIBOSE) POLYMERASE); NAD+ AD ribosyltransferase

1.65475	0.0561	0.001	250248_at	AT5G13740	ZIF1 (ZINC INDUCED FACILITATOR 1); carbohydrate transmembrane transporter/ sugar:hydrogen ion symporter
1.6524	0.06165	0.00105	249843_at	AT5G23570	SGS3 (SUPPRESSOR OF GENE SILENCING 3)
1.65155	0.051	0.0009	257634_s_at	AT3G26170;A	[AT3G26170, CYP71B19 (cytochrome P450, family 71, subfamily B, pc 19); oxygen binding];[AT3G26180, CYP71B20 (cytochrome P450, famil subfamily B, polypeptide 20); oxygen binding]
1.64475	0.06815	0.00125	262614_at	AT1G13980	GN (GNOM)
1.64245	0.06835	0.00125	254490_at	AT4G20320	CTP synthase
1.64185	0.05775	0.0011	264507_at	AT1G09415	NIMIN-3 (NIM1-INTERACTING 3)
1.63775	0.0733	0.0014	260037_at	AT1G68840	RAV2 (REGULATOR OF THE ATPASE OF THE VACUOLAR MEMBR binding / transcription factor
1.6367	0.06105	0.00105	256418_at	AT3G06160	transcriptional factor B3 family protein
1.6244	0.05955	0.00125	246253_at	AT4G37260	AtMYB73/MYB73 (myb domain protein 73); DNA binding / transcription
1.6186	0.06945	0.0013	265354_at	AT2G16700	ADF5 (ACTIN DEPOLYMERIZING FACTOR 5); actin binding
1.61055	0.07315	0.0014	257431_at	AT2G36360	kelch repeat-containing protein
1.6096	0.0714	0.0014	265057_at	AT1G52140	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT3G16330.1); hypothetical protein [Vitis vinifera] (GB:CAN64915.1)
1.6059	0.067	0.00165	246601_at	AT1G31710	copper amine oxidase, putative
1.59975	0.07715	0.0015	250864_at	AT5G03870	glutaredoxin family protein
1.59695	0.0746	0.00145	255032_at	AT4G09500	glycosyltransferase family protein
1.59165	0.07705	0.0015	262656_at	AT1G14200	zinc finger (C3HC4-type RING finger) family protein
1.5865	0.0766	0.0015	251189_at	AT3G62650	binding
1.58395	0.07355	0.0016	264101_at	AT1G79000	HAC1 (P300/CBP ACETYLTRANSFERASE-RELATED PROTEIN 2 GE H3/H4 histone acetyltransferase/ transcription cofactor
1.5827	0.07555	0.0015	255030_at	AT4G09480	transposable element gene
1.57945	0.07785	0.0016	254667_at	AT4G18280	glycine-rich cell wall protein-related
1.5571	0.0816	0.00175	265324_at	AT2G18250	ATCOAD (4-PHOSPHOPANTETHEINE ADENYLYLTRANSFERASE); nucleotidyltransferase/ pantetheine-phosphate adenylyltransferase
1.5562	0.08445	0.0018	251782_at	AT3G55260	ATHEX2/HEXO1 (BETA-HEXOSAMINIDASE 1); beta-N-acetylhexosar hexosaminidase/ hydrolase, hydrolyzing O-glycosyl compounds
1.5495	0.0836	0.002	260051_at	AT1G78210	hydrolase, alpha/beta fold family protein
1.54495	0.09435	0.00225	254901_at	AT4G11530	protein kinase family protein

Upregulated genes where there was a significant expression level difference between parents. Lowest fold change is reported only. (For all genes in this case it was Sha).

FC	pfp	P.value	Array Element	Locus Identifie	Annotation
6.2546	0	0	259385_at	AT1G13470	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G13520.1); unnamed protein product [Vitis vinifera] (GB:CAO42040.1); contains Int domain Protein of unknown function DUF1262 (InterPro:IPR010683)
2.7634	0	0	255895_at	AT1G18020;A	[AT1G18020, 12-oxophytodienoate reductase, putative];[AT1G17990, 1 oxophytodienoate reductase, putative]
3.2523	0	0	261942_at	AT1G22590	AGL87; transcription factor
4.0176	0	0	262082_s_at	AT1G56140;A AT1G56120	[AT1G56140, leucine-rich repeat family protein / protein kinase family protein];[AT1G56130, leucine-rich repeat family protein / protein kinase protein];[AT1G56120, leucine-rich repeat family protein / protein kinase protein]
3.8582	0	0	256601_s_at	AT3G28290;A	[AT3G28290, AT14A];[AT3G28300, AT14A]
3.8139	0	0	252345_at	AT3G48640	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT5G66670.2); unknown protein [Arabidopsis thaliana] (TAIR:AT5G66670.1)
2.1228	0.0026	0	245456_at	AT4G16950	RPP5 (RECOGNITION OF PERONOSPORA PARASITICA 5)
3.1606	0	0	248169_at	AT5G54610	ANK (ANKYRIN); protein binding

Supplementary Table 6. Similarity of *OAK* and related alleles.

	At5g59670 Col-0	**At5g59670a Bla-1**	**OAK Bla-1**	**At5g59670a Sha**	**OAK Sha**
At5g59670 Col-0	–	84	89	89	87
At5g59670a Bla-1	75	–	83	87	83
OAK Bla-1	81	71	–	91	94
At5g59670a Sha	83	78	85	–	95
OAK Sha	79	72	91	93	–

Nucleotide identity in percent is given on top, with amino acid identity given on bottom.

Supplementary Table 7. Survey of 87 *A. thaliana* accessions for *OAK* duplication.

Accession ID	Accession name	*OAK* duplication
CS76409	Agu-1	Yes
CS76392	Bak-2	Yes
CS76393	Bak-7	Yes
CS22591	Bor-4	Yes[a]
CS76410	Cdm-0	Yes
CS22614	Cvi-0	Yes
CS22683	Est-1	Yes
CS76423	ICE102/Galdo-1	Yes
CS76363	ICE112	Yes
CS76425	ICE120/Valsi-1	Yes
CS76426	ICE138/Leb-3	Yes
CS76379	ICE150	Yes
CS76380	ICE152	Yes
CS76381	ICE153	Yes
CS76354	ICE181	Yes
CS76355	ICE212	Yes
CS76356	ICE213	Yes
CS76349	ICE226	Yes
CS76350	ICE228	Yes
CS76419	ICE29/Slavi-1	Yes
CS76372	ICE33	Yes
CS76369	ICE36	Yes
CS76348	ICE50	Yes
CS76352	ICE79	Yes
CS76362	ICE91	Yes[a]
CS76366	ICE92	Yes
CS22651	Kondara	Yes[b]
CS22607	Kz-9	Yes
CS76390	Lag2-2	Yes
CS76413	Leo-1	Yes
CS22686	Ler	Yes
CS76388	Lerik	Yes
CS76414	Mer-6	Yes
CS76400	Star-8	Yes
CS76403	TüSB30-2	Yes

CS76391	Vash	Yes
CS76408	Wal-HäsB-4	Yes
CS22679	Bur-0	No
CS22681	Col-0	No
CS76397	Del-10	No
CS76386	Dog-4	No
CS76411	Don-0	No
CS76399	Ey 1.5-2	No
CS76412	Fei-0	No
CS76404	HKT2-4	No
CS76373	ICE1	No
CS76367	ICE104	No
CS76365	ICE106	No
CS76364	ICE107	No
CS76361	ICE111	No
CS76424	ICE119	No
CS76385	ICE127	No
CS76384	ICE130	No
CS76383	ICE134	No
CS76353	ICE163	No
CS76357	ICE169	No
CS76358	ICE173	No
CS76370	ICE21	No
CS76351	ICE216	No
CS76347	ICE49	No
CS76377	ICE60	No
CS76378	ICE61	No
CS76420	ICE63	No
CS76371	ICE7	No
CS76421	ICE70	No
CS76375	ICE71	No
CS76374	ICE72	No
CS76376	ICE73	No
CS76422	ICE75	No
CS76368	ICE93	No
CS76359	ICE97	No
CS76360	ICE98	No

CS76389	Istisu-1	No
CS76395	Kastel	No
CS76396	Koch	No
CS76398	Nemrut	No
CS76402	Nie1.2	No
CS76415	Ped-0	No
CS76416	Pre-6	No
CS76417	Qui-0	No
CS76406	Rü3.1-27	No
CS22646	Se-0	No
CS22647	Ts-1	No
CS76401	Tü-Sha-9	No
CS76407	Tü-V-12	No
CS76405	TüWa1-2	No
CS76418	Vie-0	No
CS76387	Xan-1	No
CS76394	Yeg-1	No

[a]These accessions also contain the At5g59670 Col-0 like promoter. [b]Kondara has a similar incompatibility phenotype to Sha when crossed to Bla-1. It differs by two intergenic nucleotides in the 17.5 kb *RLK* cluster, so was excluded from population structure analyses.

Supplementary Figures

Supplementary Figure 1. Bla-1/Sha incompatibility decreases seed set.

(**a**) Normal appearing Col-0 plants that are either non-transgenic or carry only a single *OAK* transgene. The phenotype of F_1 plants with both *OAK* transgenes is comparable to (**b**) Sha/Bla-1 F_1 plants. (**c**) Total seed set after three months shown as box and whisker plots. Boxes in box plot cover the first and third quartile, and the whiskers represent values that are not more than 1.5 times the interquartile range. A two-tailed, unequal variance t-test showed statistical equivalence of seed set between wild-type plants and those with a single *OAK* transgene, and highly significant reduction of seed set in plants carrying both transgenes.

Supplementary Figure 2. High humidity suppresses outgrowth formation.

Bla-1/Sha F_1 plants were grown for 3 and a half weeks under either high humidity (covered with a dome and surrounded by water), normal humidity (controlled 65% humidity), or under drought stress conditions (65% humidity but minimal watering). Two representative leaves per treatment are shown. Outgrowths are indicated by arrows.

Supplementary Figure 3. Effect of auxin and cytokinin concentration on callus formation.

Callus formation at 12 days for transverse sections of leaves and petioles of Bla-1, Bla-1/Sha F1 and Sha. Three representative tissue pieces are shown per accession and hormone concentration.

Supplementary Figure 4. Mapping interval for the Bla-1/Sha outgrowth causal gene.

(**a**) Positional cloning markers used according to the cognate genes and position in Mbp in reference accession Col-0. (**b**) The genes in reference accession Col-0 in the final mapping interval, with protein kinases marked in light grey and the RLKs highlighted in mid-grey.

Supplementary Figure 5. AmiRNA knockdown of *OAK* rescues the hybrid phenotype.

AmiRNAs designed against each RLK in the *OAK* cluster from Bla-1 (**a**) or Sha (**b**) were transformed into Bla-1/Sha F_1 plants and plants heterozygous at the RLK locus identified in the next generation. One representative plant per line is shown. Scale bar = 1 cm.

Supplementary Figure 6. Potential LRR and malectin-like domains in OAK.

(**a**) The consensus for plant-specific LRR domains is given below according to (Kobe, B. & Kajava, A.V. The leucine-rich repeat as a protein recognition motif. *Curr. Opin. Struct. Biol.* **11**, 725-32; 2001), with residues conserved in over 50% of proteins shown in uppercase. Leucine resides from OAK at conserved positions are indicated in yellow, with other conserved residues highlighted in green. Less conserved residues or residues similar to those conserved are highlighted in light grey. (**b**) Predicted malectin-like domains (Schallus, T. et al. Malectin: a novel carbohydrate-binding protein of the endoplasmic reticulum and a candidate player in the early steps of protein N-glycosylation. *Mol. Biol. Cell* **19**, 3404-14; 2008) in OAK_{Bla-1} and OAK_{Sha}. Although the amino acid sequence identity is low (11-15%), the secondary structure is more highly conserved, and the probability scores are very high.

Supplementary Figure 7. Divergence of RLK orthologs and paralogs.

(**a**) Comparison of pairwise amino acid divergence between OAK_{Bla-1} and OAK_{Sha} and between all RLKs in this cluster. (**b**) Comparison of pairwise amino acid divergence between OAK and At5g59670a alleles from Bla-1 and Sha.

Supplementary Figure 8. Compatibility between *OAK*-containing accessions.

Cytoscape (Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, et al. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13: 2498-2504) representation of crosses performed between *OAK*-containing accessions (names indicated in circles). Node color on the periphery indicates the haplotype group of the second malectin domain. Cvi-0, Cdm-0, ICE50, ICE226 and ICE228 alleles

switch between haplotype groups, and are shown in intermediate colours. Absence of color indicates that the haplotype group is not known. Compatible hybrid combinations are indicated by grey edges, and incompatible ones with outgrowths with black edges.

Supplementary Figure 9. Much of the *OAK* promoter is derived from a duplicated region of *RLK* coding sequence.

Top 15 hits from LALIGN (http://www.ch.embnet.org/software/LALIGN_form.html) are shown according to position in the Bla-1 *OAK* promoter, linked to a colour-matched box indicating position in the Col-0 *RLK* cluster.

Supplementary Figure 10. Alignment of the OAK proteins from Sha, Leo-1 and Bla-1.

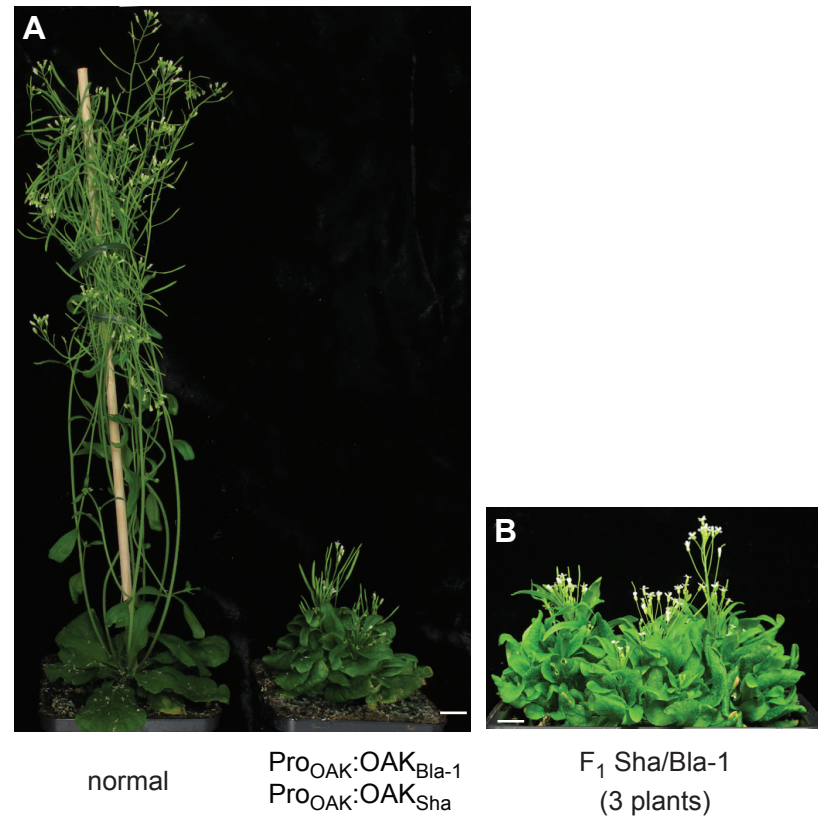
Amino acid differences between the three OAK proteins are indicated in purple (where Sha differs from Leo-1 and Bla, which are both incompatible with Sha), in cyan (where Bla-1 differs from Sha and Leo-1) and in red (where Leo-1 differs from Sha and Bla-1). Alignment was performed with CLUSTALW (Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, et al. (2003) Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res 31: 3497-3500).

Supplementary Figure 11. Expression of the OAK extracellular domain in hybrid plants can reduce the severity of aberrant phenotypes.

The extracellular domains of OAK_{Sha}, OAK_{Bla} or $At5g59670_{Col-0}$ under control of their native promoters or the 35S promoter were transformed into a segregating hybrid background and scored for the hybrid phenotype. Transformants were genotyped for allelic status at the endogenous *OAK* locus to identify heterozygous individuals. Plants with a mild phenotype where only a few outgrowths were observed on the petioles but that were otherwise phenotypically wild-type were combined with the “wild-type” category.

Supplementary Figure 12. Mis-expressed *OAK* couples to the salicylic acid signalling pathway.

(**a**) Pro_{35S}*:nahG* when introduced into $P_{At5g59670}$*:*$OAK_{Bla\text{-}1}$ $P_{At5g59670}$*:*OAK_{Sha} rescues of the cell death phenotype. (**b**) Pro_{35S}*:nahG* when introduced into P_{OAK}*:*$OAK_{Bla\text{-}1}$ P_{OAK}*:*OAK_{Sha} does not suppress the outgrowths, leaf twisting or loss of apical dominance.



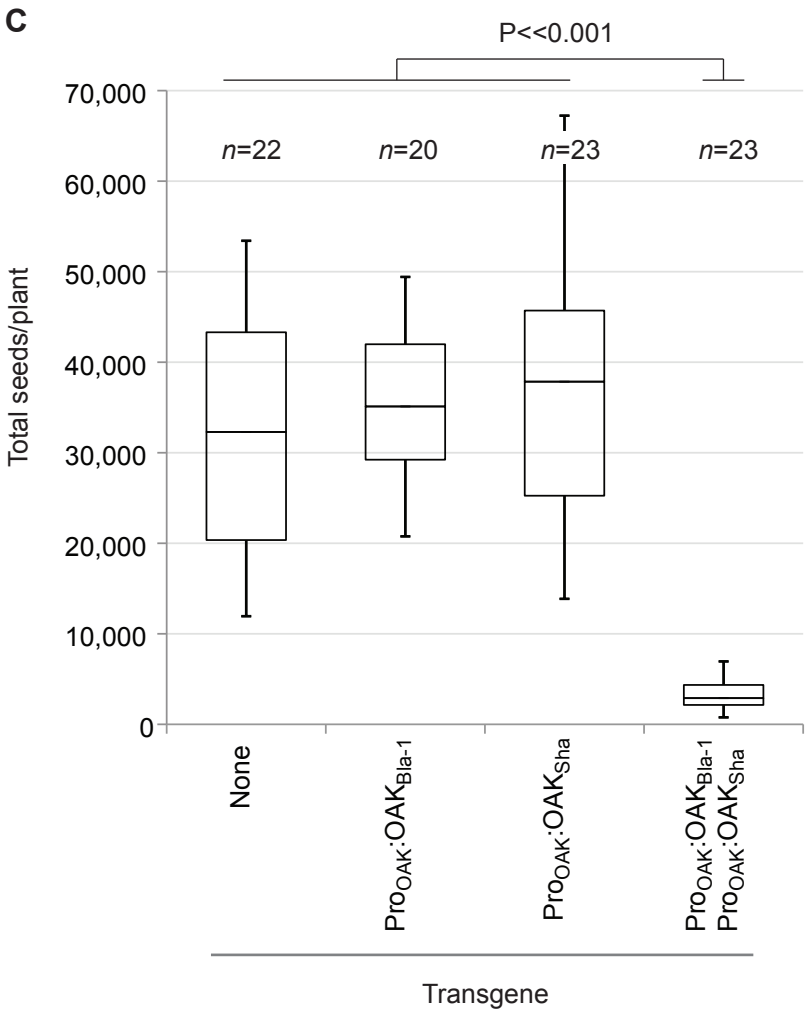
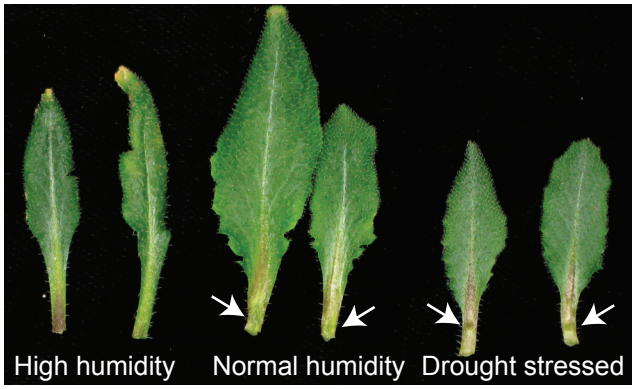
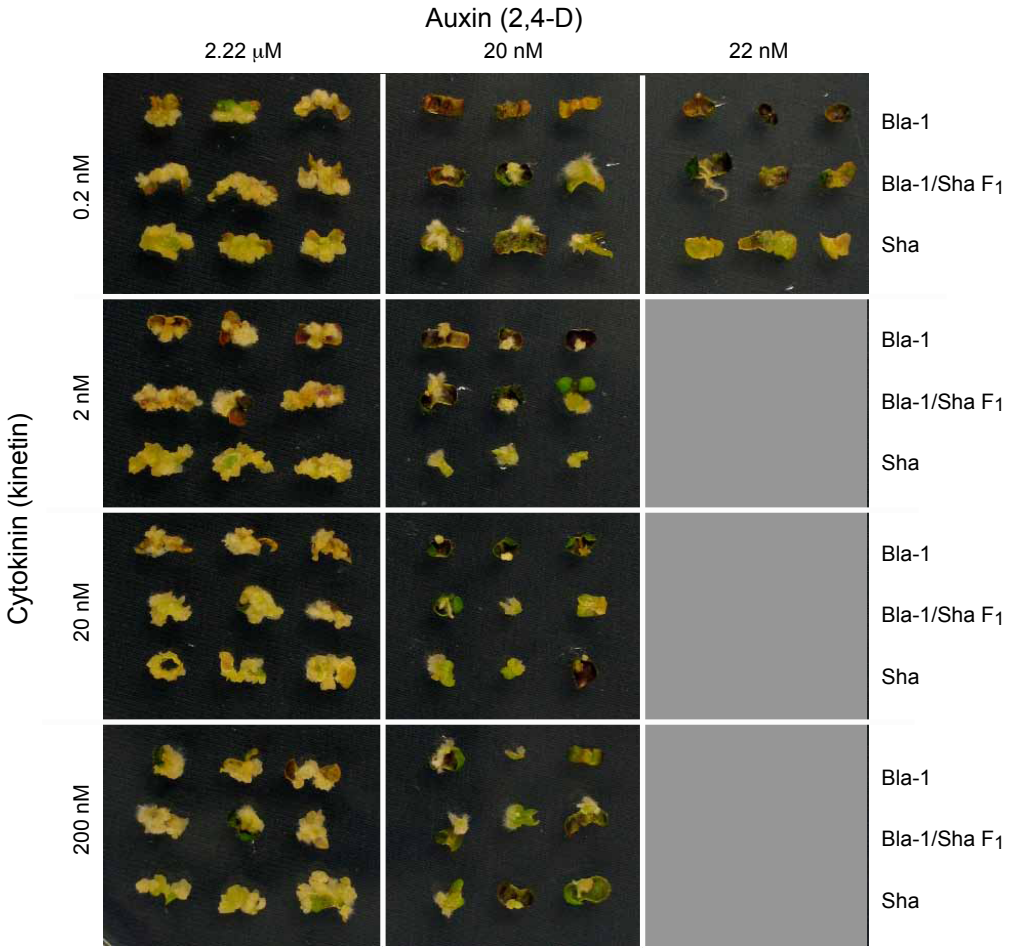


Figure S1 Smith *et al.*, 2011

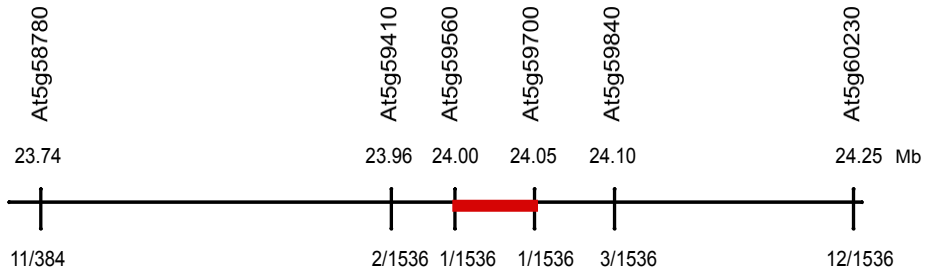


Supplementary Figure 2 Smith *et al.*, 2010



Supplementary Figure 3 Smith *et al.*, 2010

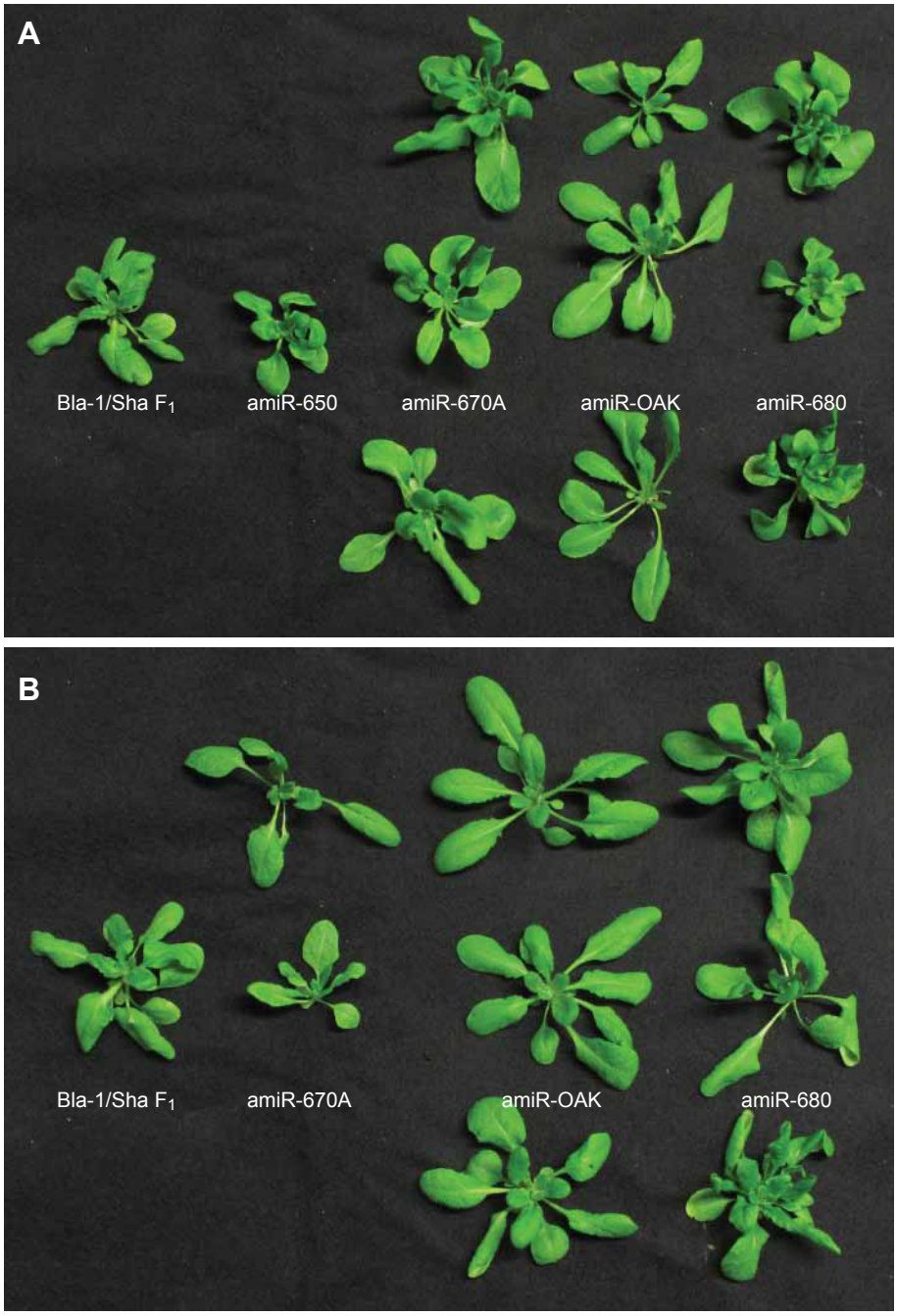
A



B

Locus	Protein
At5g59560	Sensitivity to red light reduced protein
At5g59570	Myb family transcription factor
At5g59580	UDP-glucosyl transferase family protein
At5g59590	UDP-glucosyl transferase family protein
At5g59600	pentatricopeptide (PPR) repeat-containing protein
At5g59610	DNAJ heat shock N-terminal domain-containing protein
At5g59613	Similar to unknown protein
At5g59616	Protein kinase-related
At5g59620	CACTA-like transposase family
At5g59630	Pseudogene
At5g59640	CACTA-like transposase family
At5g59650	LRR protein kinase
At5g59660	LRR protein kinase
At5g59670	LRR protein kinase
At5g59680	LRR protein kinase
At5g59690	Histone H4
At5g59700	Putative protein kinase

Supplementary Figure 4 Smith et al., 2010



Supplementary Figure 5 Smith *et al.*, 2010

A LRR domains

```
Bla-1 OAK (amino acids 409-502)
PPRITSLNLSSSR LNGTIATAIQS
LTQLETLDLSNNN LTGGVPEFLGK
MKSLSVINLSGNN LNGSIPQALRK
  KRVKLYLEGNPRLIKR PKEKIS

Sha OAK (amino acids 409-502)
PPRITSLNLSSSR LNGTIATAIQS
LTQLETLDLSNNN LTGGVPEFLGK
MKSLSVINLSGNN LNGSIPQALRK
 KRL KLYLEGNPRLIKR PKEKIP

L..L..L.L..N. L12.Ip..LG.
Where 1 = t/s
      2 = g/-
```

B Malectin-like domains

Bla-1

```
No 1
>2jwp_A Malectin, MGC80075; sugar binding, sugar binding protein; NMR {Xenopus laevis} PDB: 2k46 A*
  Probab=99.79  E-value=5.1e-19  Score=164.79  Aligned_cols=143  Identities=14%  Similarity=0.123  Sum_probs=0.0

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  Q Consensus        30 sIdCG~~~~t~~y~d~~~~w~sD~~~i~G~~~v~~~~~~~~~~~~y~t~R~F~~g~~~cY~~v~~~~yl  104 (524)
                        .||||+++...+.      ||.|.+|..|...|..............     +|+|+|.|+..  .||.||+.++++|+
  T Consensus         9 ~INcGG~~~~d~~~~~~g~~~~D~~~~~~~~~~~~~~~~~t~~~lY~T~R~~~~~~~~Y~~v~~~g~Y~   80 (174)
  T 2jwp_A            9 AVNAGGESHVDVH------GIHYRKDPLEGRVGRASDYGMKLPILRSNPEDQVLYQTERYNEDS--FGYDIPIKEEGEYV   80 (174)
  T ss_dssp             EEEETSSSEEETT------TEEECSSCSSTTCCCCCCCTTSCCSSSCHHHHHTTTCCCCCCSC--EEEEEECCSCEEEE
  T ss_pred             EEECCCCCcCCCC------CCEEeCCCCcCccccccccccccccccccccCCchhhceeeeCCCc--cEEEEEcCCCcEEE


  Q ss_pred            EEEEECCcccCCCCCCceeEEEEcceEEEE-----------------EEecCCCceEEEEEEEcCCCeEEEEEeeCCCC
  Q Wed_Aug_04_13:  105 ITASFLYGNYDGHNIAPVFDLYLGPNLWAN-----------------IDLEDVNGKWEEILHIPTSNSLQICLVKTGMA  166 (524)
  Q Consensus       105 VRl~F~ygnyd~~~~~~Fdv~~~~~~~t-----------------v~~~~~~~~E~i~~~~~~l~vcf~~~~~g  166 (524)
                        |||||....++..+. +.|||++++..+..                 ...-....++.|+...+.++.+.|||+++..+
  T Consensus        81 vrLhF~e~~~~~~~~r~Fdv~v~g~~~~~di~~~g~~~~~~~~~~~v~~~~l~~~~~~~~g~l~i~f~~~~~~  159 (174)
  T 2jwp_A           81 LVLKFAEVYFAQSQQ-KVFDVRVNGHTVVKDLDIFDRVGHSTAHDEIIPISIKKGKLSVQGEVSTFTGKLSVEFVKGYYD  159 (174)
  T ss_dssp             EEEEEECCSCCCSSS-SCEEEEETTEEEEEEECCHHHHSSSSCEEEEEEEEEETTEEEETTEEECCSEEEEEEECSSSC
  T ss_pred             EEEEEEeeccCCcCC-ccEEEEECCEEEEeecCHHHhCCCCeeEEEEEEEecCeEEEEEEecCCCcEEEEeCCCCCC


  Q ss_pred            CccceEEEEEECCCc
  Q Wed_Aug_04_13:  167 TPLISSLELRPMRTR  181 (524)
  Q Consensus       167 ~pFIs~iEl~~l~~~  181 (524)
                        .++++++++...+
  T Consensus       160 ~p~inaIEI~kg~~d  174 (174)
  T 2jwp_A          160 NPKVCALFIMKGTAD  174 (174)
  T ss_dssp             SSSEEEEEEESSCCC
  T ss_pred             CcEEEEEEEECCCC


  No 2
>2jwp_A Malectin, MGC80075; sugar binding, sugar binding protein; NMR {Xenopus laevis} PDB: 2k46 A*
  Probab=99.61  E-value=2.7e-16  Score=146.27  Aligned_cols=152  Identities=11%  Similarity=0.043  Sum_probs=0.0

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  Q Consensus       191 ~~~~R~n~G~~~~~r~p~D~~dR~W~~~~~~~~~~~~st~~~i~~~~~~~~P~~Vy~TA~~~~~~~ln~tw~~~~~  267 (524)
                        +.++|+||||+...     |..+|.|.+|...............  ....+..+|||||.....     ++|.+ ++
  T Consensus         5 ~v~~~INcGG~~~~~~~d~~g~~~~D~~~~~~~~~~~~~~~~~~~~~t~~~~lY~T~R~~~~~~~~~~Y~~v~   74 (174)
  T 2jwp_A            5 KVIWAVNAGGESHV-----DVHGIHYRKDPLEGRVGRASDYGMKLPILRSNPEDQVLYQTERYNEDS-----FGYDIPIK   74 (174)
  T ss_dssp             HEEEEEETSSSEE-----ETTTEEECSSCSSTTCCCCCCCTTSCCSSSCHHHHHTTTCCCCCCSC-----EEEEECC
  T ss_pred             cEEEEECCCCCcC-----CCCCCEEeCCCCcCccccccccccccccccccCCchhhceeeeCCc-----cEEEEEcC


  Q ss_pred            CCccEEEEEEccccccCCCceeEEEEecccccccccccccceEEEEecccccCceE-----------EEEEec
  Q Wed_Aug_04_13:  268 FDDQYYFYAHYAEIQDLQANDTREFNFLLNGQKLYVPSTEVPEKLSLTTFQSPSPTSCNGWEC-----------YFQLIR  336 (524)
  Q Consensus       268 ~~~~y~v~lHF~Ei~~~~~~R~F~IyiNg~~~~~~~~~~~l~~~~~~~~s~~~~~l-----------~~sL~~  336 (524)
                        +++.|+|||||+.....++ |+|+|++||+.+..+.++.........+.........+.+          .+.+.-
  T Consensus        75 ~g~Y~vrLhF~e~~~~~~~r~Fdv~v~g~~~~~~di~~~g~~~~~~~~~~~v~~~~l~~~~~~~~g~l~i~f   153 (174)
  T 2jwp_A           75 EEGEYVLVLKFAEVYFAQSQQ-KVFDVRVNGHTVVKDLDIFDRVGHSTAHDEIIPISIKKGKLSVQGEVSTFTGKLSVEF   153 (174)
  T ss_dssp             SCEEEEEEEEECCSCCCSSS-SCEEEEETTEEEEEEECCHHHHSSSSCEEEEEEEEEETTEEEETTEEECCSEEEEEE
  T ss_pred             CCcEEEEEEEEeccCCcCC-ccEEEECCEEEEeecCHHHhCCCCeeEEEEEEEecCeEEEEEEecCCcEEEEe


  Q ss_pred            cCCCCCchhhhhhhhhh
  Q Wed_Aug_04_13:  337 TKRSTLPPLLNALEVYT  353 (524)
  Q Consensus       337 t~~StlpPILNalEIy~  353 (524)
                        .....-.|+|||+||+|
  T Consensus       154 ~~~~~~~p~inaIEI~k  170 (174)
  T 2jwp_A          154 VKGYYDNPKVCALFIMK  170 (174)
  T ss_dssp             ECSSSCSSSEEEEEEES
  T ss_pred             CCCCCCCcEEEEEEEEE
```

Sha

```
  No 1
>2jwp_A Malectin, MGC80075; sugar binding, sugar binding protein; NMR {Xenopus laevis} PDB:  2k46  A*
  Probab=99.79  E-value=3.5e-19  Score=165.88  Aligned_cols=143  Identities=15%  Similarity=0.113  Sum_probs=0.0

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  Q Consensus         30 sIdCG~~~~~t~~~~d~~t~~~w~~D~~~i~~g~~~~v~~~~~~~~~~~-----~y~TaR~Fp~g~~~cY~~~v~~~~~yl  104 (522)
                         .||||+++...+.      ||.|.+|..|...|....+..........     +|+|+|+|+.  .+||.||+.++|+|+
  T Consensus          9 ~INcGG~~~~d~~------g~~~~~D~~~~~~~~~~~~~~~~~~~~~~~t~~~~lY~T~R~~~~--~~~Y~~~v~~~g~Y~   80 (174)
  T 2jwp_A             9 AVNAGGESHVDVH------GIHYRKDPLEGRVGRASDYGMKLPILRSNPEDQVLYQTERYNED--SFGYDIPIKEEGEYV   80 (174)
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  T ss_pred               EEECCCCCcCCCC------CCEEeCCCCcCcccccccccccccccccccCCchhhceeeecCC--ccEEEEEcCCCCcEEE


  Q ss_pred               EEEEECCcCcCCCCCCceEEEEEeeeEEEE------------------EEecCCCcEEEEEEEEcCCCcEEEEEeeCCCC
  Q Wed_Aug_04_13:   105 IRAYFLYGNYDGHNIAPVFDLYLGPNLWAK------------------IDLQDVNGKWEEILHIPTSNSLQICLVKTGMA  166 (522)
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                         |||||....++..+. +.||+++++..+..                  ...-....++.|+...+.++.+.|||+++..+
  T Consensus         81 vrLhF~e~~~~~~~~~-r~Fdv~v~g~~~~~~~di~~~~g~~~~~~~~~~~~~v~~~~l~~~~~~~~~~~g~l~i~f~~~~~~  159 (174)
  T 2jwp_A            81 LVLKFAEVYFAQSQQ-KVFDVRVNGHTVVKDLDIFDRVGHSTAHDEIIPISIKKGKLSVQGEVSTFTGKLSVEFVKGYYD  159 (174)
  T ss_dssp              EEEEEECCSCCCSSS-SCEEEEETTEEEEEEECHHHHHSSSSCEEEEEEEEEETTEEEETTEEEECCSEEEEEEECSSSC
  T ss_pred               EEEEEEeecccCCcCC-ccEEEEECCEEEEeecCHHHhcCCCCceeEEEEEEEEecCeEEEEEEEeccCCcEEEEeCCCCCC


  Q ss_pred               CcceEEEEEEEECCCc
  Q Wed_Aug_04_13:   167 TPLISSLELRPMRTG  181 (522)
  Q Consensus        167 ~pFIsaiEv~~l~d~  181 (522)
                         .||||||||.+...+
  T Consensus        160 ~p~inaIEI~kg~~d  174 (174)
  T 2jwp_A           160 NPKVCALFIMKGTAD  174 (174)
  T ss_dssp              SSSEEEEEEEESSCCC
  T ss_pred               CcEEEEEEEEEECCCC


  No 2
>2jwp_A Malectin, MGC80075; sugar binding, sugar binding protein; NMR {Xenopus laevis} PDB:  2k46  A*
  Probab=99.73  E-value=2.8e-18  Score=159.76  Aligned_cols=152  Identities=14%  Similarity=0.114  Sum_probs=0.0

  Q ss_pred               ceeEEEEEecCCCCcceeecCCCcccccccccccCCcccceeeccccCCC--CccChHHHHHhhccccCCCcceEEEEecC
  Q Wed_Aug_04_13:   189 SLKTYRRFYFNKSGSGLRSSKDVYDRTWVPFFMKEWTQISTDLGVKNDN--KYVPPEDALKTAATPTNASEPLTIKWTNS  266 (522)
  Q Consensus        189 ~L~~~~R~n~G~~~~~i~~~~D~~dR~W~p~~~~~~~~~~~st~~~~~~~~--~~~~P~~Vy~TA~~~~n~s~~lnltw~~~  266 (522)
                         |-++++|+||||....      |...|.|.+|..+...............  ....+..+|||||....      +++|.++
  T Consensus          3 a~~v~~~INcGG~~~~-----d~~g~~~~~D~~~~~~~~~~~~~~~~~~~~~~~t~~~~lY~T~R~~~~-----~~~Y~~~   72 (174)
  T 2jwp_A             3 ADKVIWAVNAGGESHV-----DVHGIHYRKDPLEGRVGRASDYGMKLPILRSNPEDQVLYQTERYNED-----SFGYDIP   72 (174)
  T ss_dssp              HHHEEEEEEETSSSEE-----ETTTEEECSSCSSTTCCCCCCCTTSCCSSSCHHHHHTTTCCCCCCS-----CEEEEEE
  T ss_pred               cccEEEEEECCCCCcC-----CCCCCEEeCCCCcCcccccccccccccccccCCchhhceeeecCC-----ccEEEEE


  Q ss_pred               CCCCccEEEEEEecccccCcCCceEEEEEECCeecccCCcCcccccceeEEEeeeEee--------------CCCcEEE
  Q Wed_Aug_04_13:   267 DNPNAQYYVYRHFAEIQDLRANDIREFNMLWNGVAMSPDPEIPTKLKVNTIYSQSPRFC--------------DEGKCIF  332 (522)
  Q Consensus        267 vd~~~~y~VrLhF~Ei~~~~~~~~~R~F~IyiNg~~~~~~~~v~~~~~~~~~~~~~~~~~~--------------~sg~~~i  332 (522)
                         ++++.+|+|||||||+.....++ |+|+|++||+.+.+++++.........+.......               .+|.+.+
  T Consensus         73 v~~~g~Y~vrLhF~e~~~~~~~~~-r~Fdv~v~g~~~~~~~di~~~~g~~~~~~~~~~~~~v~~~~l~~~~~~~~~~~g~l~i  151 (174)
  T 2jwp_A            73 IKEEGEYVLVLKFAEVYFAQSQQ-KVFDVRVNGHTVVKDLDIFDRVGHSTAHDEIIPISIKKGKLSVQGEVSTFTGKLSV  151 (174)
  T ss_dssp              CCSCEEEEEEEEEECCSCCCSSS-SCEEEEETTEEEEEEECHHHHHSSSSCEEEEEEEEEETTEEEETTEEEECCSEEEE
  T ss_pred               cCCCcEEEEEEEEEeeccCCcCC-ccEEEEECCEEEEeecCHHHhcCCCCceeEEEEEEEEecCeEEEEEEEeccCCcEEE


  Q ss_pred               EEEecCCCCcCCccccchhccc
  Q Wed_Aug_04_13:   333 QLIRTNRSTLPPLLNAFEVYT  353 (522)
  Q Consensus        333 sl~~t~~StlpPilNalEIy~  353 (522)
                         .+.++...  .|+|||+||++
  T Consensus        152 ~f~~~~~~--~p~inaIEI~k  170 (174)
  T 2jwp_A           152 EFVKGYYD--NPKVCALFIMK  170 (174)
  T ss_dssp              EEECSSSC--SSSEEEEEEEES
  T ss_pred               EeCCCCCC--CcEEEEEEEEE
```

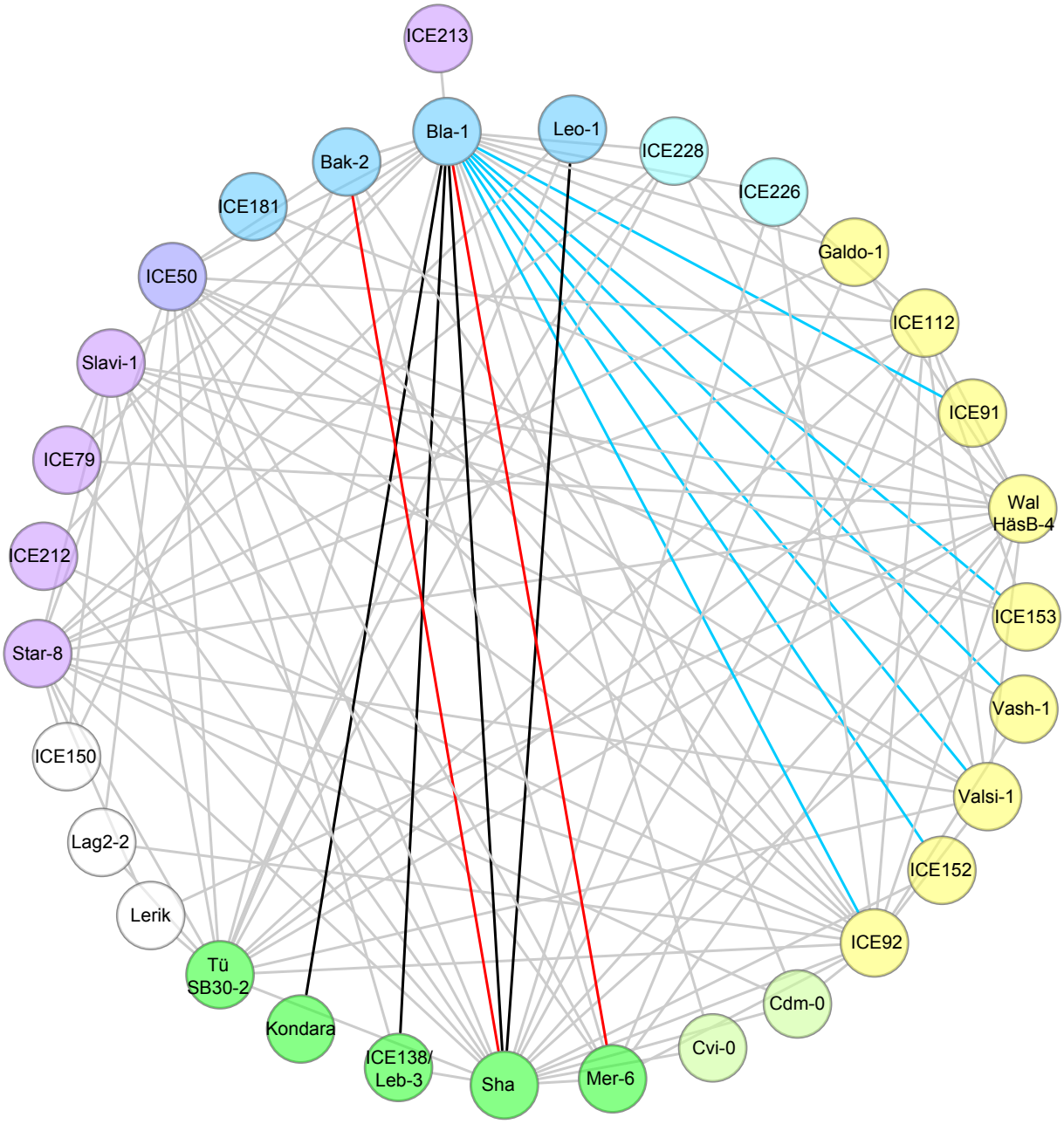
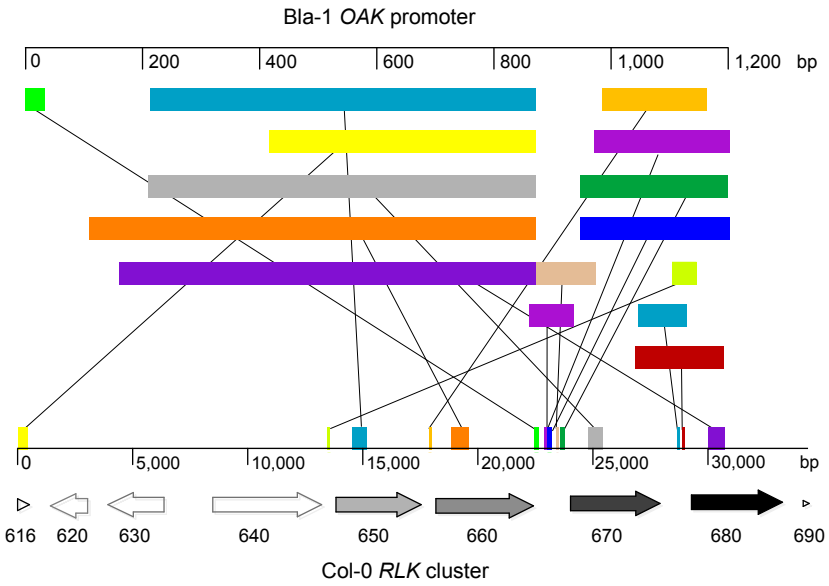


Figure S8 Smith *et al.*, 2011



Supplementary Figure 9 Smith *et al.*, 2010

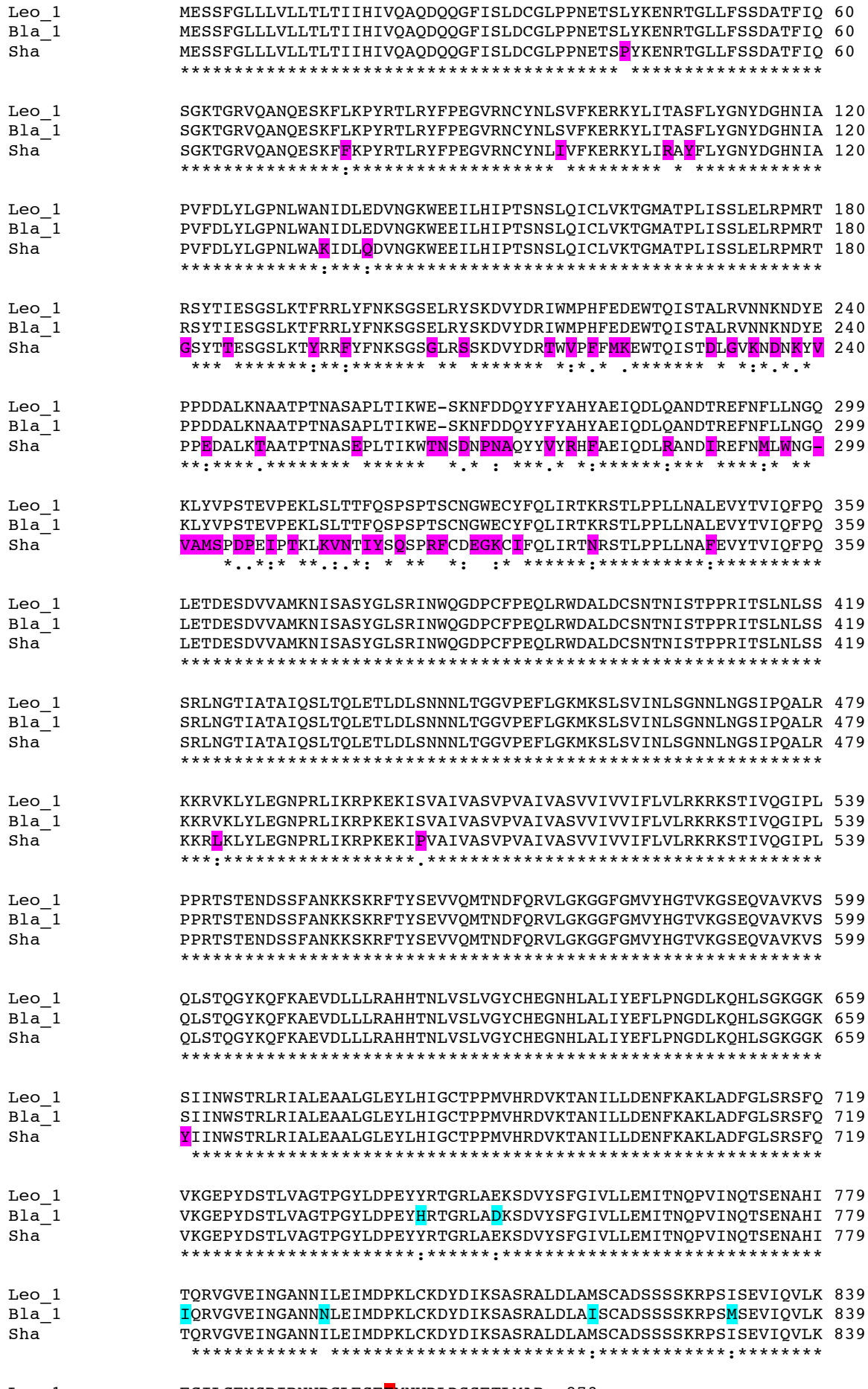
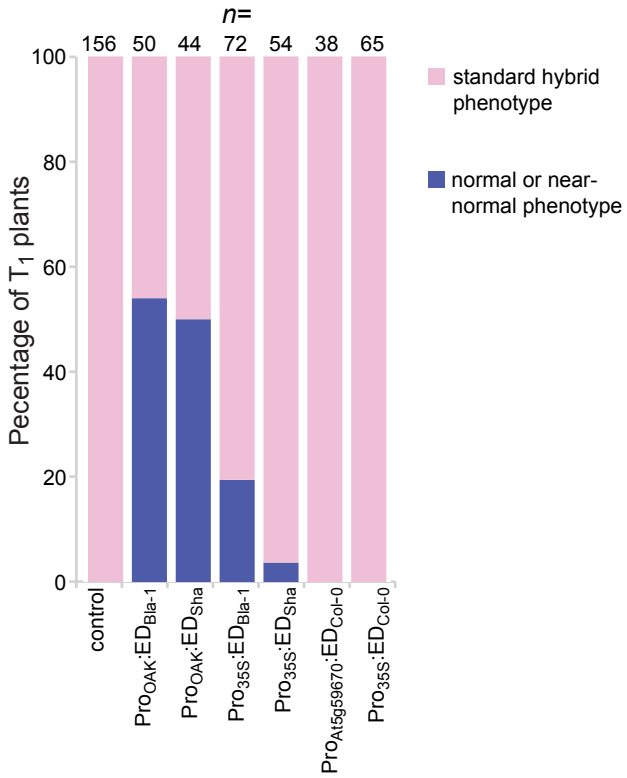
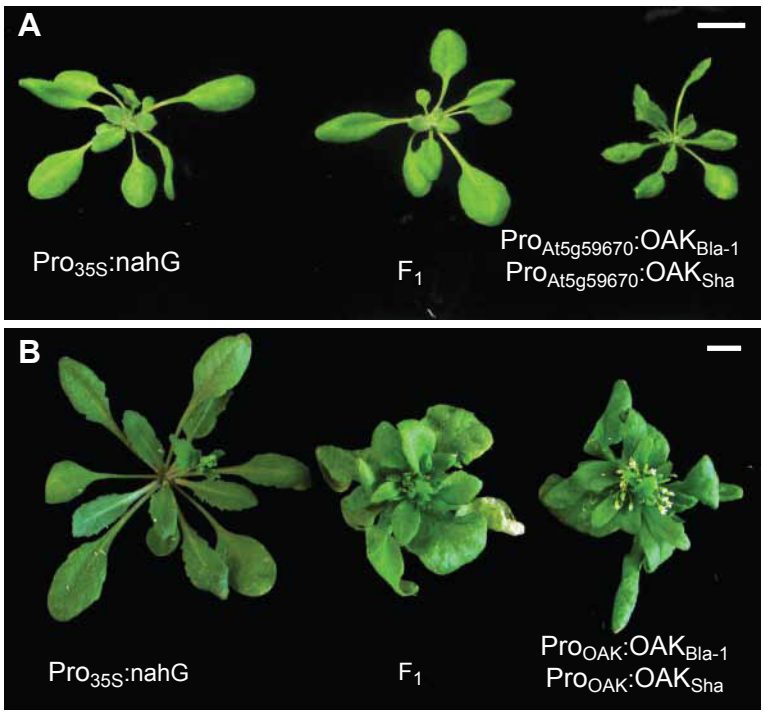


Figure S10 Smith *et al.*, 2011



Supplementary Figure 11 Smith *et al.*, 2010



Supplementary Figure 12 Smith *et al.*, 2010